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David M. Whitacre *Editor*

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Foreword

International concern in scientific, industrial, and governmental communities over traces of xenobiotics in foods and in both abiotic and biotic environments has justified the present triumvirate of specialized publications in this field: comprehensive reviews, rapidly published research papers and progress reports, and archival documentations. These three international publications are integrated and scheduled to provide the coherency essential for nonduplicative and current progress in a field as dynamic and complex as environmental contamination and toxicology. This series is reserved exclusively for the diversified literature on "toxic" chemicals in our food, our feeds, our homes, recreational and working surroundings, our domestic animals, our wildlife, and ourselves. Tremendous efforts worldwide have been mobilized to evaluate the nature, presence, magnitude, fate, and toxicology of the chemicals loosed upon the Earth. Among the sequelae of this broad new emphasis is an undeniable need for an articulated set of authoritative publications, where one can find the latest important world literature produced by these emerging areas of science together with documentation of pertinent ancillary legislation.

Research directors and legislative or administrative advisers do not have the time to scan the escalating number of technical publications that may contain articles important to current responsibility. Rather, these individuals need the background provided by detailed reviews and the assurance that the latest information is made available to them, all with minimal literature searching. Similarly, the scientist assigned or attracted to a new problem is required to glean all literature pertinent to the task, to publish new developments or important new experimental details quickly, to inform others of findings that might alter their own efforts, and eventually to publish all his/her supporting data and conclusions for archival purposes.

In the fields of environmental contamination and toxicology, the sum of these concerns and responsibilities is decisively addressed by the uniform, encompassing, and timely publication format of the Springer triumvirate:

Reviews of Environmental Contamination and Toxicology [Vol. 1 through 97 (1962–1986) as Residue Reviews] for detailed review articles concerned with

any aspects of chemical contaminants, including pesticides, in the total environment with toxicological considerations and consequences.

Bulletin of Environmental Contamination and Toxicology (Vol. 1 in 1966) for rapid publication of short reports of significant advances and discoveries in the fields of air, soil, water, and food contamination and pollution as well as methodology and other disciplines concerned with the introduction, presence, and effects of toxicants in the total environment.

Archives of Environmental Contamination and Toxicology (Vol. 1 in 1973) for important complete articles emphasizing and describing original experimental or theoretical research work pertaining to the scientific aspects of chemical contaminants in the environment.

Manuscripts for Reviews and the Archives are in identical formats and are peer reviewed by scientists in the field for adequacy and value; manuscripts for the Bulletin are also reviewed, but are published by photo-offset from camera-ready copy to provide the latest results with minimum delay. The individual editors of these three publications comprise the joint Coordinating Board of Editors with referral within the board of manuscripts submitted to one publication but deemed by major emphasis or length more suitable for one of the others.

Coordinating Board of Editors

Preface

The role of *Reviews* is to publish detailed scientific review articles on all aspects of environmental contamination and associated toxicological consequences. Such articles facilitate the often complex task of accessing and interpreting cogent scientific data within the confines of one or more closely related research fields.

In the nearly 50 years since *Reviews of Environmental Contamination and Toxicology* (*formerly Residue Reviews*) was first published, the number, scope, and complexity of environmental pollution incidents have grown unabated. During this entire period, the emphasis has been on publishing articles that address the presence and toxicity of environmental contaminants. New research is published each year on a myriad of environmental pollution issues facing people worldwide. This fact, and the routine discovery and reporting of new environmental contamination cases, creates an increasingly important function for *Reviews*.

The staggering volume of scientific literature demands remedy by which data can be synthesized and made available to readers in an abridged form. Reviews addresses this need and provides detailed reviews worldwide to key scientists and science or policy administrators, whether employed by government, universities, or the private sector.

There is a panoply of environmental issues and concerns on which many scientists have focused their research in past years. The scope of this list is quite broad, encompassing environmental events globally that affect marine and terrestrial ecosystems; biotic and abiotic environments; impacts on plants, humans, and wildlife; and pollutants, both chemical and radioactive; as well as the ravages of environmental disease in virtually all environmental media (soil, water, air). New or enhanced safety and environmental concerns have emerged in the last decade to be added to incidents covered by the media, studied by scientists, and addressed by governmental and private institutions. Among these are events so striking that they are creating a paradigm shift. Two in particular are at the center of everincreasing media as well as scientific attention: bioterrorism and global warming. Unfortunately, these very worrisome issues are now superimposed on the already extensive list of ongoing environmental challenges.

The ultimate role of publishing scientific research is to enhance understanding of the environment in ways that allow the public to be better informed. The term "informed public" as used by Thomas Jefferson in the age of enlightenment conveyed the thought of soundness and good judgment. In the modern sense, being "well informed" has the narrower meaning of having access to sufficient information. Because the public still gets most of its information on science and technology from TV news and reports, the role for scientists as interpreters and brokers of scientific information to the public will grow rather than diminish. Environmentalism is the newest global political force, resulting in the emergence of multinational consortia to control pollution and the evolution of the environmental ethic.Will the new politics of the twenty-first century involve a consortium of technologists and environmentalists, or a progressive confrontation? These matters are of genuine concern to governmental agencies and legislative bodies around the world.

For those who make the decisions about how our planet is managed, there is an ongoing need for continual surveillance and intelligent controls to avoid endangering the environment, public health, and wildlife. Ensuring safety-in-use of the many chemicals involved in our highly industrialized culture is a dynamic challenge, for the old, established materials are continually being displaced by newly developed molecules more acceptable to federal and state regulatory agencies, public health officials, and environmentalists.

Reviews publishes synoptic articles designed to treat the presence, fate, and, if possible, the safety of xenobiotics in any segment of the environment. These reviews can be either general or specific, but properly lie in the domains of analytical chemistry and its methodology, biochemistry, human and animal medicine, legislation, pharmacology, physiology, toxicology, and regulation. Certain affairs in food technology concerned specifically with pesticide and other food-additive problems may also be appropriate.

Because manuscripts are published in the order in which they are received in final form, it may seem that some important aspects have been neglected at times. However, these apparent omissions are recognized, and pertinent manuscripts are likely in preparation or planned. The field is so very large and the interests in it are so varied that the editor and the editorial board earnestly solicit authors and suggestions of underrepresented topics to make this international book series yet more useful and worthwhile.

Justification for the preparation of any review for this book series is that it deals with some aspect of the many real problems arising from the presence of foreign chemicals in our surroundings. Thus, manuscripts may encompass case studies from any country. Food additives, including pesticides, or their metabolites that may persist into human food and animal feeds are within this scope. Additionally, chemical contamination in any manner of air, water, soil, or plant or animal life is within these objectives and their purview.

Manuscripts are often contributed by invitation. However, nominations for new topics or topics in areas that are rapidly advancing are welcome. Preliminary communication with the editor is recommended before volunteered review manuscripts are submitted.

Summerfield, NC, USA David M. Whitacre

Contents

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Chiral Pesticides: Identification, Description, and Environmental Implications

Elin M. Ulrich, Candice N. Morrison, Michael R. Goldsmith, and William T. Foreman

Contents

1 Introduction: Molecular Asymmetry in Pesticides

Anthropogenic chemicals, including pesticides, are a major source of contamination and pollution in the environment. Pesticides have many positive uses: increased food production, decreased damage to crops and structures, reduced disease vector popu-

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Table 1 Glossary for terms related to chirality (IUPAC 2006)

Achiral—not chiral; capable of being superimposed on its mirror image
Atropisomers—a subset of enantiomers which arise from restricted rotation about a single bond
Axis of chirality—an axis within a molecule with moieties attached that creates nonsuperimposable mirror images
Chiral—an object/molecule which is nonsuperimposable on its mirror image and has no mirror plane, center of inversion, or rotation–reflection axis
Chiral carbon—a carbon atom that is attached to four different moieties. This is a specific case of a chiral center
Chiral or stereogenic center—an atom with moieties attached that creates nonsuperimposable mirror images
Diastereomers—stereoisomers that are not mirror images
Enantiomers—a pair of molecular forms that are mirror images and nonsuperimposable
Meso-compounds—achiral forms of a set of stereoisomers/diastereomers that also includes chiral forms
Plane of chirality—a planar chemical unit connected by a rotationally restricted bond such that symmetry is not possible
Racemic—equal amounts of a pair of enantiomers
Stereoisomers—forms of a molecule that differ only in the arrangement of their atoms in space

lations, and more. Nevertheless, pesticide exposure can pose risks to humans and the environment, so various mitigation strategies are exercised to make them safer, minimize their use, and reduce their unintended environment effects. One strategy that may help achieve these goals relies on the unique properties of chirality or molecular asymmetry. Some common terms related to chirality are defined in Table 1.

The likelihood of introducing new pesticides to the marketplace that contain multiple chiral centers and unresolved mixtures of stereoisomers has increased. One reason is because more natural products and their derivatives have become the source of inspiration for designing new pesticides, and the molecular structure of these compounds has become increasingly complex (Williams 1997). The trend toward more complex structures that have multiple chiral centers also has occurred in the pharmaceutical industry (Feher and Schmidt 2003). There is one major difference in how these chemicals are developed and produced: chiral drugs are routinely tested, and often intentionally marketed as individual stereoisomers, whereas pesticides generally are not (Williams 1996; Stanley and Brooks 2009).

If a single stereoisomeric form contains all of the desired pesticidal properties (i.e., the biologically active stereoisomer or eutomer), but an unresolved racemic (equal amounts) formulation of the stereoisomers is applied, then at least twofold more is applied than is necessary (Ariëns 1989). Specifically, if N chiral elements exist in the pesticide molecule, then there are 2^N maximum possible stereoisomers. If only one of the 2^N stereoisomers exhibits exclusive or dominant pesticidal activity, then the percent excess of minimally or inactive stereoisomers (distomers) in the applied material will be $100 \times (2^N - 1)/2^N$, a 50% excess of distomer(s) for $N = 1$, a 75% excess for $N = 2$, etc. Although lacking intended pesticidal activity, any distomer(s) in the applied pesticide material may nevertheless have detrimental environmental consequences (e.g., to nontarget organisms). Development and manufacturing processes that enrich the eutomer and minimize or eliminate the distomer(s)

in formulated pesticides may, therefore, reduce chemical contamination, thus representing a more sustainable approach with ecosystems and environmental benefits.

Careful consideration of molecular structure is also important for scientists conducting pesticide environmental fate and effect studies, because substantial differences exist in the biological activity of distinct stereoisomers and their mixtures. Stereoisomers can degrade at different rates in the environment and in organisms, primarily through biological processes (Müller and Kohler 2004). Selective degradation of stereoisomers can alter risk factors because it potentially produces differences in exposure, toxicity, and bioavailability. Fundamental to any research conducted on stereoisomers is the need to apply stereospecific analytical methods, especially chiral separation techniques, to characterize the asymmetric pesticide components that may exist in environmental and exposure studies. If nonchiral chemical analysis techniques are used in environmental or toxicological assessments, the unique properties of individual stereoisomers in pesticide material are completely missed (Müller and Buser 1997). Many researchers have long treated isomers as discrete compounds that have unique properties; yet to this day, stereoisomers often are neglected in this regard.

This chapter is intended for use by hands-on researchers like chemists, toxicologists, environmental scientists, and modelers who wish to study the stereoisomers of environmental contaminants, particularly chiral pesticides. To assist researchers who are new to the application of chiral properties and principles, we address common stereochemical elements, provide identification of chiral pesticides, and indicate which have had potential enantioselective analysis techniques already developed for them. With this knowledge, we hope that more researchers will incorporate stereoisomer considerations into their repertoire. Furthermore, in this review, we list all known chiral pesticides in tabular and graphical formats and provide information on known enantioselective separation methodologies.

2 Methodology: Chiral Pesticide Dataset Curation

A list of pesticide active ingredients was compiled from two primary sources: the Pesticide Manual (electronic version 4.0/paper version 14) published by the British Crop Protection Council (Mann 2006), and the Compendium of Pesticide Common Names website (http://www.alanwood.net/pesticides/) maintained by Alan Wood (Wood 1995–2010). The Pesticide Manual contained 1,524 main and superseded product entries, which included chemical and biological active ingredients used for the control of pests in crops, animals, and for public health. The Compendium website contained 1,867 chemical entries and is updated several times a year. The cutoff date for entries on our list was December 2008. The pesticides listed in these two references were reduced to 1,693 by eliminating duplications, grouping multiple names for the same compound, and grouping salts and esters with the parent compound.

If the two primary source references had identical chemical structure(s) for a given pesticide, the structure(s) were drawn using ChemBioDraw (CambridgeSoft

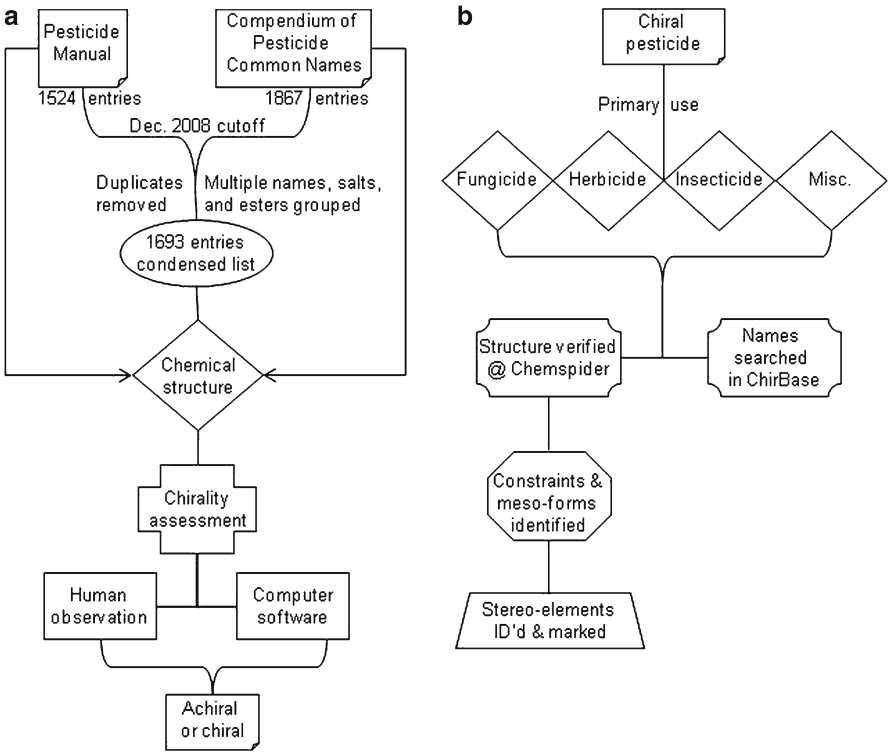


Fig. 1 Dataset curation, sources of information, and compilation procedure. (**a**) Dataset construction and chemical structure assessment. (**b**) Chiral pesticide processes

1986–2007) or ChemSketch (Advanced Chemistry Development 1994–2010). Additional internet sources, such as CambridgeSoft's ChemBioFinder website [http://www.chemfinder.com/; (CambridgeSoft 2010)] were consulted until a majority consensus regarding structure was reached. The structures were saved as .cdx and .tif files. Assessment of molecular chirality was made, first by the authors observing the structure, who specifically looked for chirality elements such as axes, centers, and planes as defined by the International Union of Pure and Applied Chemistry (IUPAC 2006). Second, this determination was confirmed by computer software. In brief, the simplified molecular input line entry specification (SMILES) codes for all of the chiral pesticides were imported into Molecular Operating Environment [MOE; (Chemical Computing Group 2010)]. The "chiral" descriptor was used to output the number of stereogenic centers detected for each molecule entered in MOE database view. This algorithm does not tend to identify atropisomerism or heteroatomic stereogenic centers (such as chiral P or S). Discrepancies between the authors' observations and computer software method were resolved by agreement among the authors. Figure 1a shows a general schematic of the processes used for the construction and for chemical structure analysis.

The chiral pesticides were grouped by primary use type: fungicide, herbicide, insecticide, or miscellaneous. These grouping were also used in the text, tables, and figures. Chiral structures were compared with structures primarily from the ChemSpider website, a free source of structure-based chemistry information [http://www.chemspider.com/; (Royal Society of Chemistry 2008)] for quality control purposes. Each chiral structure was scrutinized to identify *meso*-compounds and constrained chiral centers (see Sect. 3 for further explanation). The indications of chirality: *, †, and curved arrows were added manually to each compound, based on visual inspection of the molecule (see Sect. 3). Figure 1b shows a schematic of the processes used for each chiral pesticide.

ChirBase is the largest repository of enantioselective chromatographic separations. Three ChirBase databases, LC, GC, and CE [liquid and gas chromatography, capillary electrophoresis; (Koppenhoefer et al. 1993, 1994; Advanced Chemistry Development 1997–2010)] were searched for each chiral pesticide name. ChirBase does not contain records for every published separation; however, it is the most logical place to search for an extensive list of compounds. Any record that contained the pesticide name was considered as being a possible method. Evaluation of the records for duplication, and success of the separation method were not undertaken during this review. The methods resident in ChirBase, generally, are published in the literature and likely are replicable with the information provided.

3 Stereochemistry, Structures, and Names

Science involving the geometry and symmetry of molecules is a complex subject, and is beyond the scope of this review, although it is in the reader's best interest to become familiar with the Cahn–Ingold–Prelog rules for stereochemical notation (Cahn et al. 1966). Interested readers are directed to organic chemistry textbooks and writings for a comprehensive discussion of the subject (Mislow 1965; Nasipuri 1991; Eliel et al. 1994).

Briefly, a chiral center (or stereogenic center, or center of molecular asymmetry) occurs when four unique functional groups are bonded to an sp^3 hybridized center (i.e., with a tetrahedral geometry), which usually results in an asymmetric (chiral) molecule (Nasipuri 1991; IUPAC 2006). This geometric configuration occurs most commonly when the chiral center is a carbon atom, and results in nonsuperimposable mirror-image stereoisomers that are referred to as enantiomers (see Fig. 2a). A lone pair of electrons on an atom in the second or higher row of the periodic table (S, P, As, etc.) can act as a fourth functional group (see Fig. 2b), and produce a noncarbon chiral center that similarly is configurationally stable and produces enantiomers (Nasipuri 1991). In the figures of this review, we identify chiral centers with an asterisk (*) in the figures.

Hindered rotation about a bond can result in a unique form of molecular asymmetry known as dissymmetry (or atropisomerism or axial chirality), in which no specific chiral center gives rise to asymmetry, but rather the molecule as a whole is

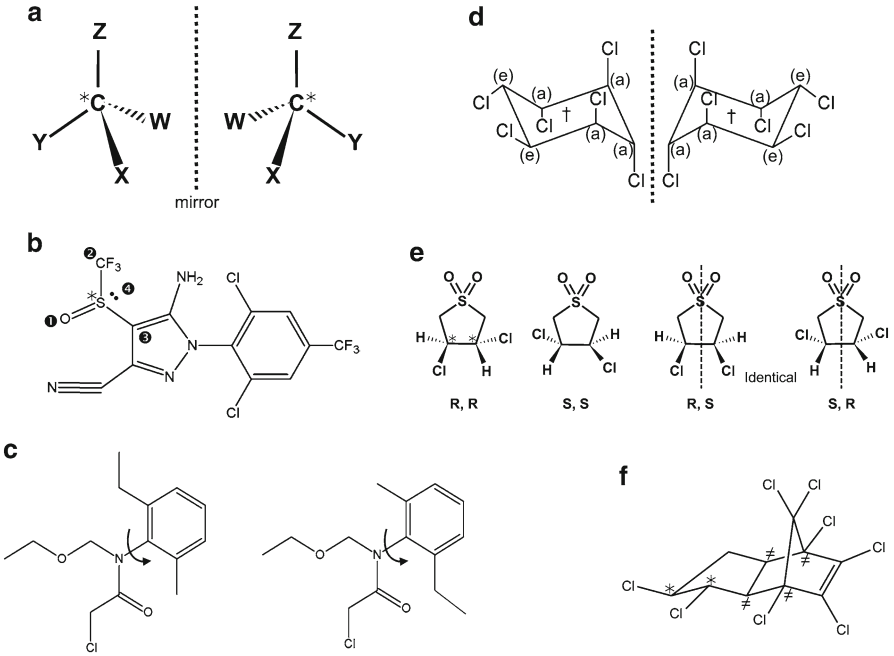


Fig. 2 Examples of the types of chirality (**a**–**d**) and special cases (**e**–**f**) encountered in pesticides (as noted in Tables 2–5 and Figs. 3–6). (**a**) Chiral center at carbon is noted by an *asterisk* (*) in structures. (**b**) Chiral sulfur atom is noted by an *asterisk* (*) in structures. Example: Fipronil. (**c**) Atropisomers caused by hindered rotation about a bond. Atropisomers are noted by a *curved arrow* in structures. Example: Acetochlor. (**d**) No plane or point of symmetry is noted by a *dagger* (†) in structures. Example: α-Hexachlorocyclohexane, a = axial, e = equatorial. The chair configuration and asymmetric chlorine placement cause chirality in this molecule. (**e**) *Meso* compounds are noted in the tables. Example: 3,4-Dichlorotetrahydrothiophene-1,1-dioxide has *R*,*R* and *S*,*S* enantiomers; but *R*,*S* and *S*,*R* are identical (superimposable) structures and constitute the *meso*-compound. (**f**) Constrained fused rings are noted in the tables. Example: Chlordane has two unconstrained (*) and four constrained (≠) chiral centers, thus four possible stereoisomers, i.e., two *cis*- and two *trans*-enantiomers

chiral (Nasipuri 1991; IUPAC 2006). These conditions result in stereoisomers that are also nonsuperimposable mirror images, and, thus constitute a pair of enantiomers (Eliel et al. 1994). Atropisomers that are reportedly stable at room temperature are identified in the figures with a curved arrow at the hindered bond (see Fig. 2c). Another type of chirality arises from the lack of a plane or point of symmetry within the molecule (see Fig. 2d). This type of chirality is prevalent in cyclic compounds and is noted in the figures by a dagger (†). Some pesticides have multiple chiral centers (*N*) and produce a theoretical maximum of 4, 8, 16, or even more different stereoisomers (2^N).

Some specific molecules that contain multiple chiral centers and diastereoisomers (stereoisomers not related as enantiomers) will have less than 2^N stereoisomers, because they contain superimposable *meso*-compounds (see Fig. 2e) (IUPAC 2006).

This situation often arises as a result of a symmetric structure in which the two halves of the molecule have an opposite configuration (Wade 1991). Pesticides that have *meso*-structures will be identified as such in the tables. Structures containing chiral centers at the junction of fused ring systems (see Fig. 2f) often have constrained geometries, resulting in intolerable bond angles and unstable configurations (Mislow 1965). Pesticides that have constrained chiral centers will be identified as such in the tables. Both *meso*-compounds and constrained geometries will reduce the total number of stereoisomers possible to less than the 2^N theoretical maximum.

Pesticide structures in the figures are drawn in a generic, nonspecific format, because multiple isomers (not due to chirality) exist for some compounds (e.g., *cis*/*trans*-isomers for the pyrethroid insecticides). When a pesticide contains multiple compounds, all structures are shown for completeness, regardless of whether they are chiral or not. When the main component of a pesticide is achiral (e.g., *p,p'*-DDT, *d*ichloro*d*iphenyl*t*richloroethane), but an isomer is chiral (e.g., *o,p'*-DDT), the main, most recognizable name and structure are used, with a note of explanation added about the chiral form.

Wherever possible, we have used the International Organization for Standardization (ISO) or other approved, recommended common or trivial names of pesticides in this review to promote easy recognition and brevity. Some additional names and abbreviations also are provided in the tables; however, trade names typically are not included. Pesticide development codes are provided for several pesticides for which a common name has only recently been, or is not yet, established. Both the figures and tables have been alphabetized using a Microsoft® sort that places numbers before letters.

All Chemical Abstract Service (CAS®) registry numbers found that were related to a given compound have been included. This may reflect unique CAS® numbers for different formulations (technical mixtures), isomers (*cis*/*trans*), salts, esters, stereochemistries, or for other reasons. Because CAS® numbers are provided, official CAS® registry names generally are not included in this review.

4 Identification and Discussion

4.1 Fungicides

In Table 2, we list 97 chiral fungicides, and show their structures in Fig. 3. In general, these fungicides have one to four chiral centers. The antibiotic subgroup of fungicides (aureofungin, blasticidin-S, cycloheximide, griseofulvin, kasugamycin, natamycin, polyoxin, and validamycin) has very complex structures that typically are prepared using semisynthetic strategies. These strategies use some starting materials that have defined absolute stereochemistry, reducing the number of possible stereoisomeric permutations; a nice feature, because these complex structures contain numerous chiral centers. Although the structure for aureofungin was not located

Table 2 Chiral fungicides

Fungicide name	Chiral features (constrained)	*Meso*?[a]	Alternate names, isomers	Salts, esters	CAS® #s	CE[b]	GC	LC
(*RS*)-*N*-(3,5-Dichlorophenyl)-2-(methoxymethyl) succinimide	1				81949-88-4			
1-Chloro-2-nitropropane	1				2425-66-3			
3-(4-Chlorophenyl)-5-methylrhodanine	1				6012-92-6			
Aldimorph	2	Y	2,5-Dimethyl	Several alkyl chains possible	91315-15-0			
Ampropylfos	1				16606-64-7			
Aureofungin	Many				8065-41-6, 63278-45-5, 63278-44-4			
Benalaxyl	1		Benalaxyl-M		71626-11-4, 98243-83-5			27
Benthiavalicarb	2			-Isopropyl	413615-35-7, 177406-68-7	3		
Bitertanol	2		Baycor		70585-36-3, 70585-38-5, 55179-31-2			1
Blasticidin-S	4			Benzylaminobenzene-sulfonate salt	2079-00-7, 51775-28-1			
Bromuconazole	2				116255-48-2			
Butylamine	1		*sec*-Butylamine		13952-84-6,13250-12-9, 513-49-5			
Captafol	2 (2)	Y	Merpafol, difolatan		2425-06-1, 2939-80-2			
Captan	2 (2)	Y			133-06-2			
Carpropamid	3				104030-54-8, 127641-62-7, 127640-90-8			
CGA 80000	1		Cloxylacon, clozylacon		79555-80-9, 67932-85-8			4
Chloraniformethan	1		Chloraniformethane		20856-57-9			

Chlozolinate	1				84332-86-5, 72391-46-9		
Climbazole	1				38083-17-9		
Cycloheximide	4				66-81-9	2	
Cyproconazole	2				94361-06-5, 94361-07-6		9
Cyprofuram	1				69581-33-5		
Dehydroacetic acid[c]	1			-Sodium	520-45-6, 771-03-9, 16807-48-0, 4418-26-2		
Diclobutrazol	2				75736-33-3, 66345-62-8		22
Diclocymet	2				139920-32-4	2	
Difenoconazole	2				119446-68-3		
Diflumetorim	1				130339-07-0		
Diniconazole	1		-M, R-		83657-24-3, 76714-88-0, 83657-18-5, 83657-19-6	3	69
Dinocap	1		DPC, 2,6-*d*initro-4-octyl*p*henyl *c*rotonates	Meptyldinocap, -4, -6	131-72-6, 39300-45-3		2
Dodemorph	2	Y		-Acetate, -benzoate	1593-77-7, 31717-87-0, 59145-63-0		
Epoxiconazole	2				133855-98-8, 135319-73-2		24
ESBP	1		*O-E*thyl *S-b*enzyl *p*henylphosphonothioate		21722-85-0		
Etaconazole	2				60207-93-4		
Ethaboxam	1				162650-77-3		54
Ethylmercury 2, 3-dihydroxypropyl mercaptide	1				2597-92-4		
Famoxadone	1				131807-57-3		
Fenamidone	1				161326-34-7		
Fenapanil	1				61019-78-1		
Fenarimol	1				60168-88-9		

(continued)

Table 2 (continued)

Fungicide name	Chiral features (constrained)	*Meso*?[a]	Alternate names, isomers	Salts, esters	CAS® #s	CE[b]	GC	LC
Fenbuconazole	1				114369-43-6			
Fenitropan	2				65934-94-3, 65934-95-4			
Fenoxanil	2		AC 382042		115852-48-7			
Fenpropidin	1				67306-00-7			2
Fenpropimorph	3				67564-91-4, 67306-03-0	1		2
Flutriafol	1				76674-21-0			9
Fosetyl	1			-Aluminum	15845-66-6, 39148-24-8			
Furalaxyl	1		-M		57764-08-6, 57646-30-7			
Furametpyr	1				123572-88-3			
Furconazole	2		*cis*-		112839-33-5, 112839-32-4			
Gliotoxin	4 (3)				67-99-2			
Griseofulvin	2				126-07-8	1		
Hexaconazole	1				79983-71-4			37
Hexylthiofos	1				41495-67-4	1		
Imazalil	1		Enilconazole	-Sulfate, -nitrate	35554-44-0, 58594-72-2, 33586-66-2			8
Ipconazole	3				125225-28-7			
Iprovalicarb	2				140923-17-7, 140923-25-7			
Isopyrazam	3 (2)				881685-58-1			
Kasugamycin	8			-Hydrochloride hydrate	6980-18-3, 19408-46-9			
Mandipropamid	1				374726-62-2	1		
Metalaxyl	1		Mefenoxam, -M		57837-19-1, 70630-17-0		4	34
Metconazole	2				125116-23-6			
Mildiomycin	6				67527-71-3			
Milneb	4	Y	Thiadiazine		3773-49-7			
Mucochloric anhydride	2	Y			4412-09-3	1		

Myclobutanil	1				88671-89-0		7
Myclozolin	1				54864-61-8		
Natamycin	14		Pimaricin		7681-93-8		
Nuarimol	1				63284-71-9		
Ofurace	1				58810-48-3		
Oxpoconazole	1			-Fumarate	134074-64-9, 174212-12-5		
Pefurazoate	1				101903-30-4	1	
Penconazole	1				66246-88-6		
Penthiopyrad	1				183675-82-3		
Piperalin	1		Pipron		3478-94-2		
Polyoxins	Var. 4+; shown 8		Polyoxin B/D,	Polyoxorim (-zinc)	11113-80-7, 19396-06-6, 22976-86-9, 146659-78-1		
Procymidone	2 (2)	Y			32809-16-8	1	
Propiconazole	2				60207-90-1		9
Propineb	1				12071-83-9, 9016-72-2		
Prothioconazole	1				178928-70-6		
Sedaxane	2		*cis*-, *trans*-		874967-67-6, 599194-51-1, 599197-38-3		
Simeconazole	1				149508-90-7		
Spiroxamine	1		*cis*-, *trans*-, or -A, -B		118134-30-8		
SSF-109	2		4-Chlorophenyl-2-(1H-1,2,4-triazol-yl) cycloheptanol		129586-32-9	1	14
Tebuconazole	1				107534-96-3	1	23
Tetraconazole	1				112281-77-3		9
Thicyofen	1				116170-30-0	2	
Triadimefon	1				43121-43-3	7	21
Triadimenol	2		-A, -B		55219-65-3, 89482-17-7, 82200-72-4		32

(continued)

Table 2 (continued)

Fungicide name	Chiral features (constrained)	*Meso*?[a]	Alternate names, isomers	Salts, esters	CAS® #s	CE[b]	GC	LC
Triarimol	1				26766-27-8			
Trichlamide	1				70193-21-4			
Tridemorph	2	Y			81412-43-3, 24602-86-6			
Triforine	2	Y			26644-46-2			
Triticonazole	1				131983-72-7			
Validamycin	14		-A		37248-47-8			
Vinclozolin	1				50471-44-8		2	12
Zarilamid	1				84527-51-5			
Zoxamide	1				156052-68-5			

[a]Y in the "*Meso*?" column indicates that yes, that pesticide has a *meso* compound

[b]The last three columns give the number of entries found in the capillary electrophoresis (CE), gas chromatography (GC), and liquid chromatography (LC) databases of ChirBase. These abbreviations are used in all tables

[c]One of the three tautomers of dehydroacetic acid has a chiral carbon

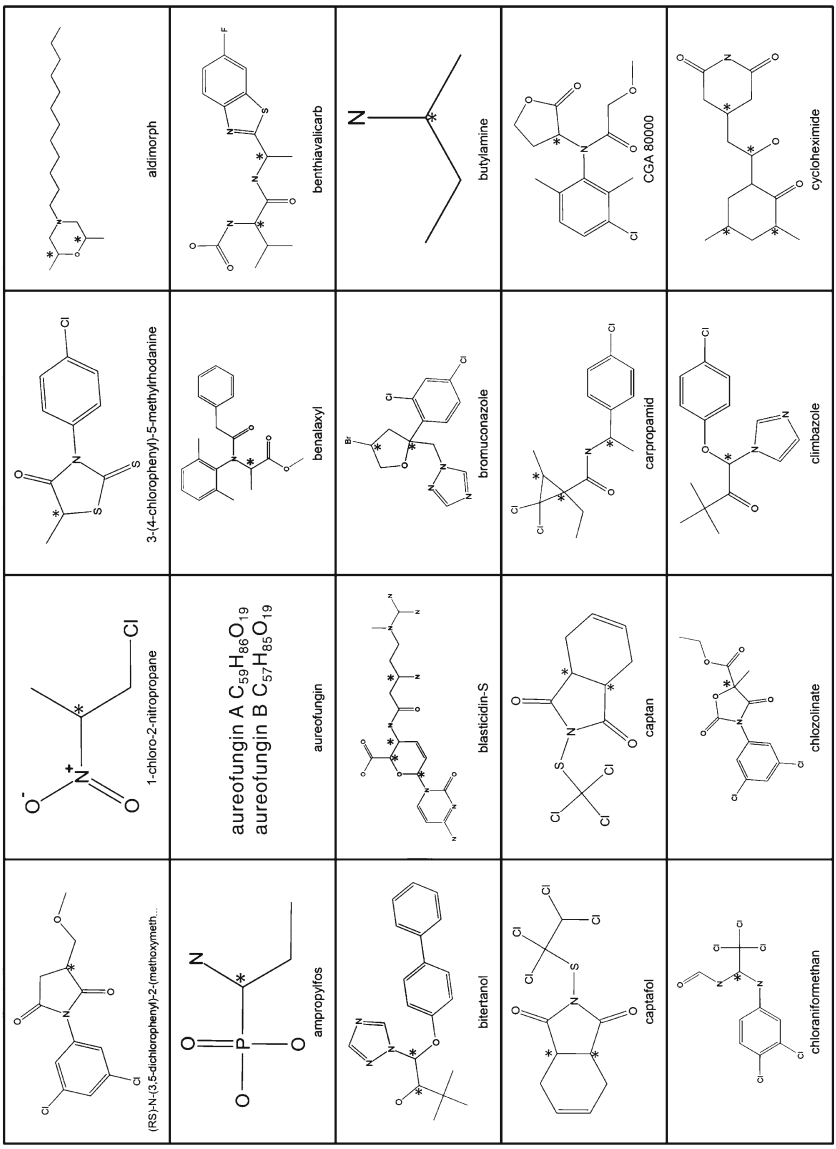


Fig. 3 Chiral fungicide structures. See Fig. 2 for symbol description

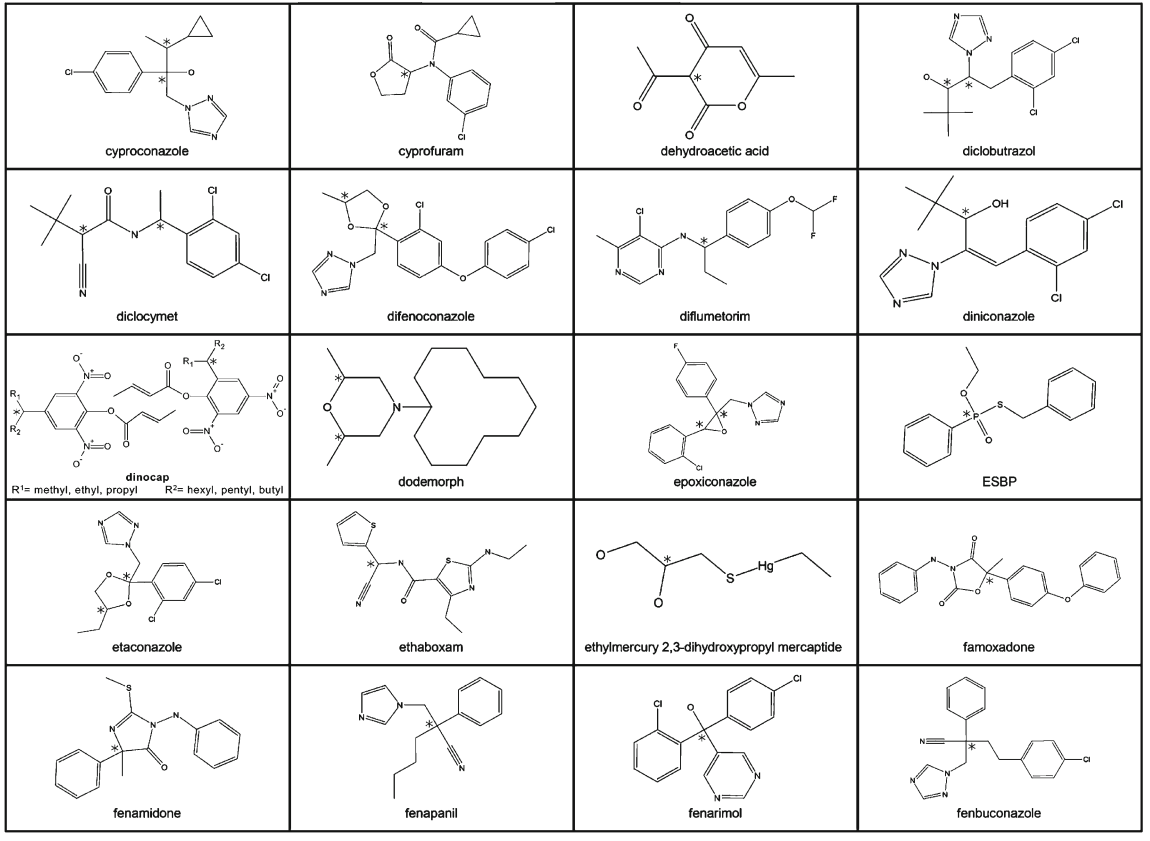


Fig. 3 (continued)

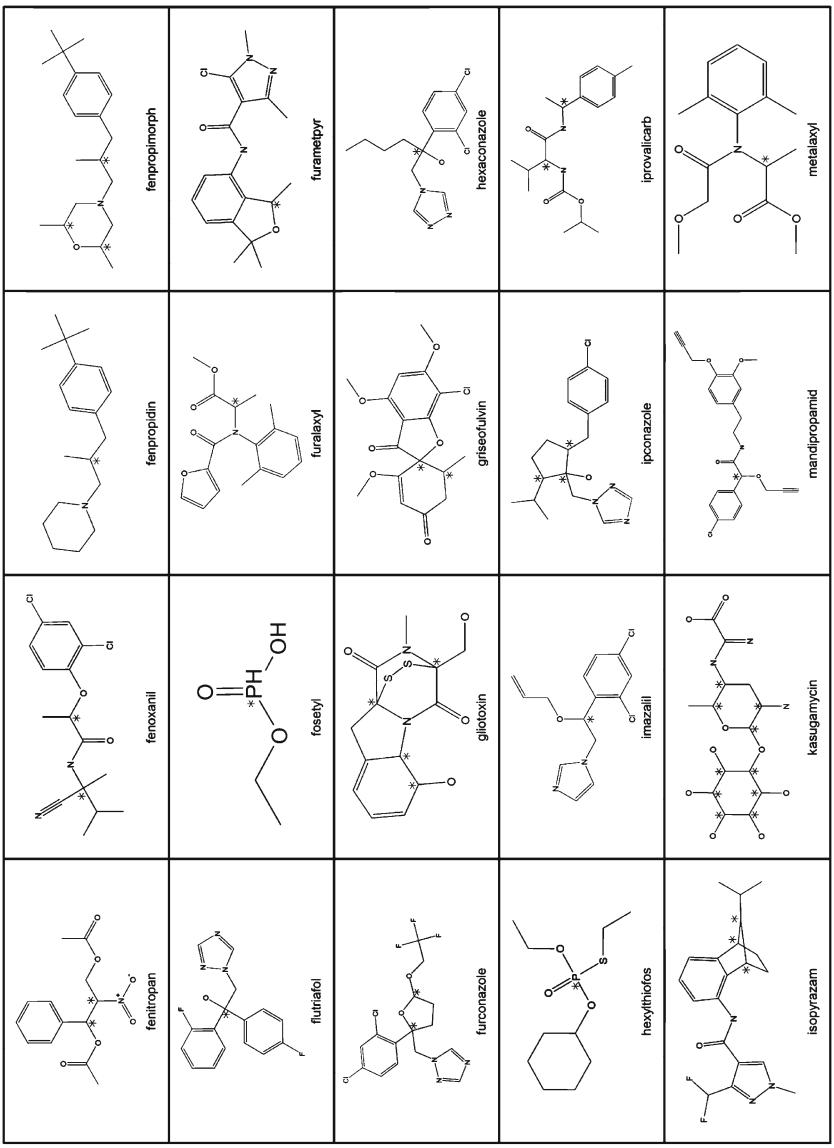


Fig. 3 (continued)

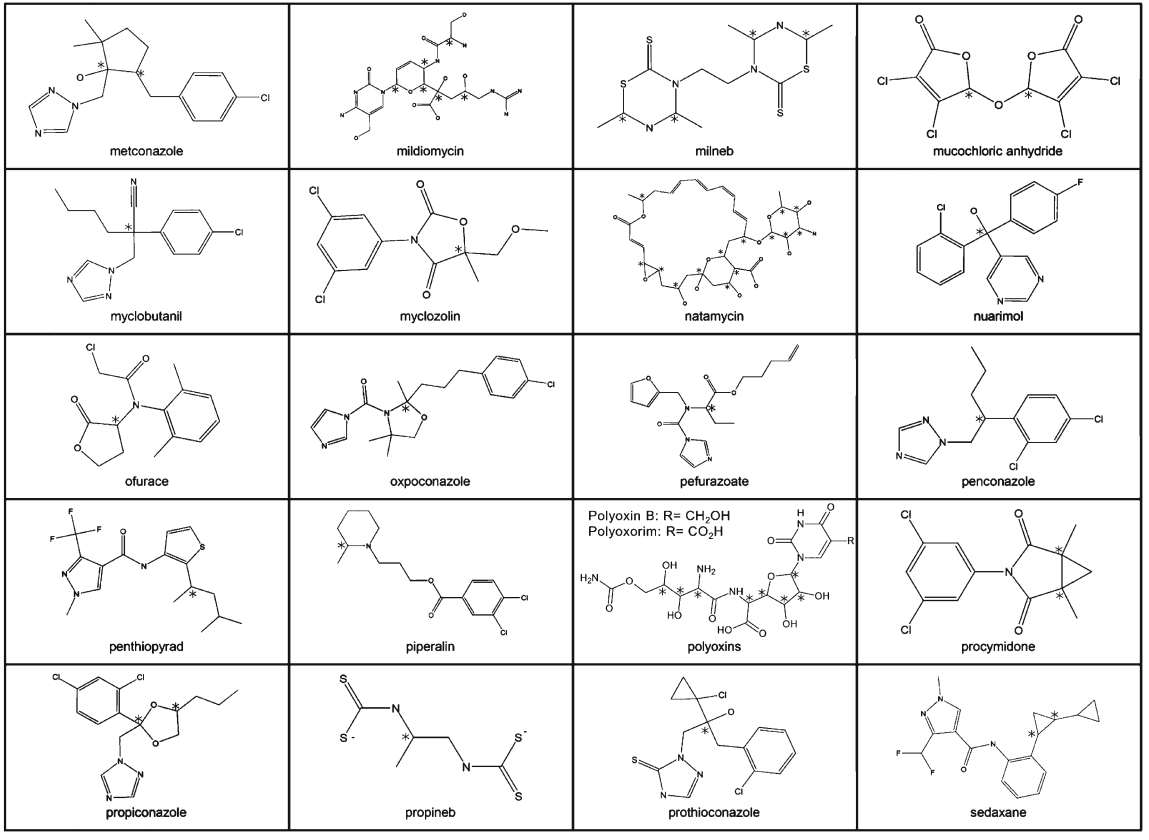
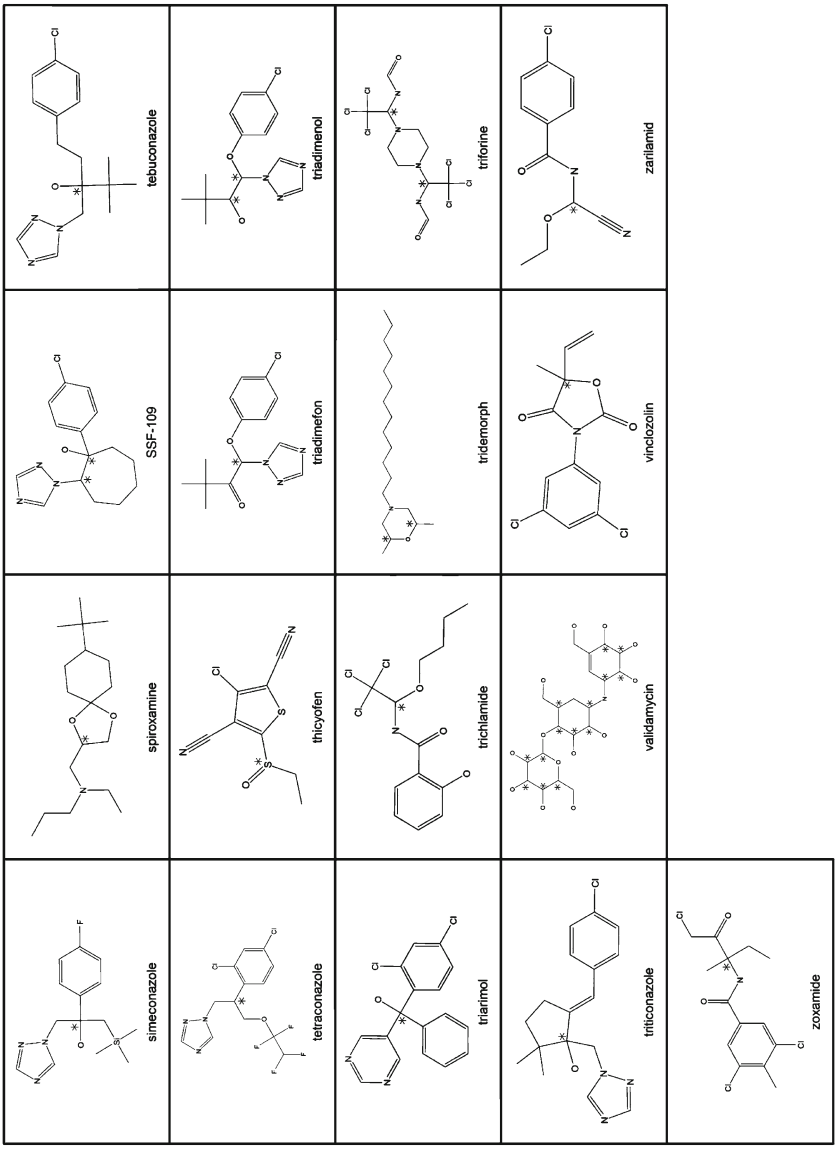


Fig. 3 (continued)


tebuconazole
triadimenol
triforine
zarilamid
SSF-109
triadimefon
tridemorph
vinclozolin
spiroxamine
thicyofen
trichlamide
validamycin
simeconazole
tetraconazole
triarimol
triticonazole
zoxamide

during our searches, it is undoubtedly chiral and was included because it contains more than 50 carbon atoms and has a close relationship to other chiral antibiotic fungicides. Dehydroacetic acid can undergo keto–enol tautomerization, and although the keto form is usually favored, the equilibrium for this compound may lie with an achiral tautomer. ESBP (common name for *O-e*thyl *S-b*enzyl *p*henylphosphonothioate), fosetyl, and hexylthiofos each have a chiral phosphorus atom, and thicyofen has a chiral sulfur atom. Sulfur has a lone pair of electrons that can act as a fourth "group," which, combined with three other unique functional groups, creates a chiral center. Heteroatomic stereogenic centers (i.e., chiral S, P, etc., but not C) tend not to be encoded frequently in stereoisomeric identification codes, such as the one used from MOE (Chemical Computing Group 2010), because these moieties are more commonly encountered in agrochemical functionalities than in drug design.

In Fig. 3, all possible chiral centers have been marked, but restrictions in ring configurations (e.g., fused rings; see Fig. 2f) may yield fewer stereoisomers. Compounds that have such constraints include captafol, captan, gliotoxin, isopyrazam, and procymidone. Constrained chiral centers are enumerated in Table 2, along with the existence of *meso* forms for nine fungicides. Ring constraints and *meso* forms need to be considered when calculating the number of possible forms prior to undertaking the analysis of stereoisomers.

There are additional reasons for compounds having fewer than the maximum possible number of stereoisomers, and these must be recognized and understood prior to analysis. Because bacteria often are used to produce the antibiotic fungicides, many of these chemicals are probably produced as one stereoisomer rather than as a mixture. Additionally, at least four of the fungicides are manufactured as single or enriched stereoisomer formulations, which may allow them to be applied at lower rates and/or lower cost (Williams 1992). They include benalaxyl, diniconazole, furalaxyl, and metalaxyl and are denoted with -M under the "alternate name, isomer" column in Table 2.

Many chiral fungicides act as ergosterol biosynthesis inhibitors and offer both protective and curative properties (Fuchs 1988). Other classes of fungicides were developed closely with herbicides that were structurally similar. Interestingly, some of the chiral fungicides also exhibit herbicidal properties, usually with a specific enantiomer displaying either herbicidal or fungicidal action (Burden et al. 1987). Paclobutrazol is one example of a compound that has dual herbicide/fungicide activity. Such compounds are listed by their primary use category as presented in the Pesticide Manual (Mann 2006).

In general, there is a wide assortment of fungicidal compounds, and the enantiomer antifungal efficacy of these compounds can vary greatly. One of the more notable compounds is triadimefon, which is converted by fungi into the fungicidally active metabolite triadimenol. In this conversion process, an additional chiral center is formed, and the four resulting stereoisomers are produced in different amounts by different species (Deas et al. 1984a, b, 1986). Fungal species can develop resistance to fungicides, making continual development of new active compounds important. This also leads to developing more complex molecules that have a greater likelihood for chiral centers.

ChirBase, the enantioselective separations database, contains entries for 25 of the 97 fungicides (26%). There are 465 records for these 25 pesticides (431 in LC, 6 in GC, and 28 in CE databases). Diniconazole has the most entries, with 72 total, and 69 of those are found in the LC database. The number of entries found for each fungicide in each ChirBase database is listed in Table 2. As previously mentioned, ChirBase does not include every published enantioselective separation, but has the most comprehensive information available. The fungicides fenarimol, hexaconazole, imazalil, myclobutanil, nuarimol, penconazole, propiconazole, and tebuconazole have no GC entries in ChirBase, but their GC separations are addressed by Bicchi et al. (1999).

4.2 Herbicides

In Table 3, we list 141 chiral herbicides, herbicide safeners, and plant growth regulators and show their structures in Fig. 4, with all possible chiral centers marked. The majority of the compounds have only 1 or 2 chiral features, whereas brassinolide and epocholeone each have 13. Acetochlor, metolachlor, and propisochlor have a bond with restricted rotation leading to atropisomers, which typically are not identified by the MOE software. Amiprofos-methyl, bilanafos, butamifos, DMPA (*O*-2,4-*d*ichlorophenyl *O*-*m*ethyl isopropyl*p*hosphor-*a*midothioate), fosamine, and glufosinate have a chiral phosphorous atom, and NC-330 has a chiral sulfur atom. Prototropy (transfer of a proton) between the =O and –OH on the chiral phosphorus atom of glufosinate may be rapidly equilibrated under certain pH conditions, rendering the tautomers difficult if not impossible to distinguish by enantioselective techniques. Restrictions in ring configurations may yield smaller numbers of stereoisomers for bicyclic compounds such as benzobicyclon, brassinolide, cinmethylin, dicyclonon, dikegulac, endothal, epocholeone, gibberellic acid, gibberellins, heptopargil, isonoruron, noruron, profluazol, and tetcyclacis. Six herbicides have more than one chiral center, but also a plane of symmetry resulting in a *meso*-compound, which reduces the number of actual stereoisomers to less than 2^N.

At least 13 of the herbicides are produced as single or enriched stereoisomer formulations. These are noted with S-, -P, or -M in the "Alternate names, isomers" or "Salts, esters" columns in Table 3, and include (*S*)-carvone, dichlorprop-P, diclofop-P-methyl, dimethenamid-P, fenoxaprop-P, flamprop-M, fluazifop-P, glufosinate-P, haloxyfop-P, mecoprop-P, *S*-metolachlor, quizalofop-P, and uniconazole-P. Many of these enriched herbicides belong to the aryloxy- or phenoxy-propionic families wherein the *R*-enantiomer exhibits the herbicidal activity (Haga et al. 1998). A "chiral switch" occurs when the manufacturer changes from a racemic formulation to an enantioenriched one. After the introduction of enantioenriched *S*-metolachlor to the commercial market, the shifting enantiomer signature of metolachlor was monitored in Swiss lake water samples over a 2-year period using enantioselective analysis (Buser et al. 2000).

Table 3 Chiral herbicides (H), plant growth regulators (PGR), and herbicide safeners (HS)

Herbicide name	Chiral features (constrained)	*Meso*?	Alternate names, isomers	Salts, esters	CAS® #s	Type	CE	GC	LC
2,4-D[a]	Varies[a]	Varies[a]	(*2,4-D*ichlorophenoxy) acetic acid	Multiple[b] *-2-butoxypropyl, -2-ethylhexyl, -lvxiancaolin, -meptyl, -tefuryl, triisopropanol-ammonium*[a]	Multiple[b] 94-75-7, 1320-18-9, 1928-43-4, 215655-76-8, 1917-97-1, 18584-79-7, 32341-80-3	H	2		
3,4-DP	1		2-(*3,4-D*ichlorophenoxy) *p*ropanoic acid		3307-41-3	H			
4-CPP	1		2-(*4-C*hloro*p*henoxy) *p*ropanoic acid		3307-39-9	H			2
Abscisic acid	1				21293-29-8	PGR	3	4	48
Acetochlor	1				34256-82-1	H	2	4	17
Alloxydim	1			-Sodium	55634-91-8, 55635-13-7	H			
Amiprofos	1			-Methyl	33857-23-7, 36001-88-4	H			
Ancymidol	1				12771-68-5	PGR			38
Aviglycine	1		AVG, *a*minoethoxy*v*inyl*g*lycine	-Hydrochloride	49669-74-1, 55720-26-8	PGR			
BCPC	1		*B*utan-2-yl (3-*c*hloro*p*henyl)*c*arbamate		2164-13-8	H			
Beflubutamid	1				113614-08-7	H			
Benoxacor	1				98730-04-2	HS			3
Benzfendizone	2				158755-95-4	H			
Benzobicyclon	2 (2)				156963-66-5	H			
Benzoylprop	1			-Ethyl	22212-56-2, 22212-55-1	H			1
Bilanafos	4		Bialaphos	-Sodium	35597-43-4, 71048-99-2	H			
Brassinolide	13 (6)			-Ethyl	72962-43-7, 74174-44-0	PGR			
Bromacil	1			-Lithium, -sodium	314-40-9, 53404-19-6, 69484-12-4	H	2	2	37
Bromobonil	1				25671-46-9	H			

Bromobutide	1				74712-19-9	H				
Butamifos	2				36335-67-8	H				
Buthidazole	1				55511-98-3	H				
Butralin	1				33629-47-9	H				
Butroxydim	1				138164-12-2	H				
Buturon	1				3766-60-7	H				2
Carbetamide	1				16118-49-3	H				2
Carfentrazone	1			-Ethyl ester	128621-72-7, 128639-02-1	H				1
Carvone	1		(*S*)-, *d*-		99-49-0, 2244-16-8, 6485-40-1	PGR	1	76	14	
Chlorazifop	1			-Propargyl	60074-25-1, 74310-70-6, 72492-54-7, 72492-55-8, 72280-52-5, 74267-69-9	H				
Chlorbufam	1				1967-16-4	H	1			1
Chlorfenprop	1			-Methyl	14437-20-8, 59604-10-3, 59604-11-4	H				
Chlorfluren	1			-Methyl	24539-66-0, 22909-50-8	PGR				
Chlorflurenol	1		Chlorflurecol	-Methyl	2464-37-1, 2536-31-4	PGR				
Chlorprocarb	1				23121-99-5	H				
Cinmethylin	3 (2)				87818-31-3, 87818-61-9, 87819-60-1	H				
Ciobutide	1				80544-75-8	PGR				
Cisanilide	2	Y			34484-70-0	H				
CL 304,415	1		4-(Carboxymethyl)-4-chromanecarboxylic acid		31541-57-8	HS				
Clethodim	2				99129-21-2	H				2
Clodinafop	1			-Propargyl	114420-56-3, 105512-06-9, 105511-96-4	H				1

(continued)

Table 3 (continued)

Herbicide name	Chiral features (constrained)	*Meso*?	Alternate names, isomers	Salts, esters	CAS® #s	Type	CE	GC	LC
Clofop	1			-Isobutyl	59621-49-7, 26129-32-8, 51337-71-4	H			
Clomeprop	1				84496-56-0	H			
Cloprop	1				101-10-0	PGR			
Cloproxydim	2				95480-33-4	H			
Cloquintocet[c]	1			***-Mexyl***[c]	88349-88-6, 99607-70-2	HS			
CPPC	1		2-Chloro-1-methylethyl 3-chlorocarbanilate		2150-32-5	H			
Cycloxydim	2				101205-02-1, 99434-58-9	H			
Cyhalofop	1			-Butyl	122008-78-0, 122008-85-9	H			
*Di*chloral*u*rea	2	Y	DCU		116-52-9	H			
Dichlorprop	1		2,4-DP, -P, 2-(*2,4-D*ichlorophen oxy)*p*ropionic acid	-Isoctyl, -butotyl, (P)-dimethylammonium, (P)-potassium, -P-sodium, -ethylammonium, -2-ethylhexyl, -methyl	7547-66-2, 120-36-5, 28631-35-8, 15165-67-0, 104786-87-0, 113963-87-4, 119299-10-4, 53404-31-2, 53404-32-3, 79270-78-3, 57153-17-0, 5746-17-8, 39104-30-8	H	13	5	50
Diclofop	1			-(P)-Methyl	40843-25-2, 51338-27-3, 71283-65-3, 75021-72-6	H	5		49
Dicyclonon	1 (1)				79260-71-2, 110499-25-7, 110499-27-9	HS			
Difenopenten	1			-Ethyl	81416-44-6, 71101-05-8	H			
Dikegulac	4 (4)		Diprogulic acid	-Sodium	18467-77-1, 52508-35-7	PGR			

Dimethametryn	1				22936-75-0	H				
Dimethenamid	1		-P		87674-68-8, 163515-14-8	H		8		
Dinofenate	1				61614-62-8	H				
Dinosam	1				4097-36-3	H				
Dinoseb	1		Binapacryl	-Acetate, -ammonium, -diolamine, -sodium, -trolamine, 3-methyl-2-butenoate	88-85-7, 2813-95-8, 6365-83-9, 53404-43-6, 35040-03-0, 6420-47-9, 485-31-4	H		2		
DMPA	1		*O*-2,4-*D*ichlorophenyl *O*-*m*ethyl isopropyl*p*hosphor-*a*midothioate		299-85-4	H				
EBEP	2	Y	*E*thyl *bi*s(2-*e*thylhexyl)*p*hosphinate		Not listed	H				
Endothal	4 (2)		Endothall	-Diammonium, -dipotassium, -disodium, -mono(*N*,*N*-dimethylalkylammonium)	145-73-3, 28874-46-6, 17439-94-0, 2164-07-0, 129-67-9, 66330-88-9	H				
Epocholeone	13 (6)				162922-31-8	PGR				
Esprocarb	1				85785-20-2	H				
Ethofumesate	1				26225-79-6	H	1		22	
Ethoxyfen	1		HC-252	-Ethyl	188634-90-4, 131086-42-5	H				
Etnipromid	1				76120-02-0	H				
Fenoprop	1		Silvex, 2,4,5-TP, 2-(*2,4,5-T*richl orophenoxy)*p*ropionic acid	-Butotyl,-butometyl, -3-butoxypropyl, -butyl, isoctyl, -methyl, -potassium	93-72-1, 6047-17-2, 13557-98-7, 4841-20-7, 2818-16-8	H	8	3	4	
Fenoxaprop	1		-P	-(P)-Ethyl	95617-09-7, 82110-72-3, 66441-23-4, 73519-55-8, 113158-40-0, 71283-80-2	H	5		33	

(continued)

Table 3 (continued)

Herbicide name	Chiral features (constrained)	*Meso*?	Alternate names, isomers	Salts, esters	CAS® #s	Type	CE	GC	LC
Fenthiaprop	1		Fentiaprop	-Ethyl	95721-12-3, 73519-50-3, 93921-16-5, 66441-11-0	H			
Flamprop	1		-M	(M)-Methyl, -(M)-isopropyl	58667-63-3, 52756-25-9, 52756-22-6, 90134-59-1, 57353-42-1, 63782-90-1, 57973-67-8, 63729-98-6	H	5		4
Fluazifop	1		-P	(P)-Butyl, -methyl	69335-91-7, 69806-50-4, 83066-88-0, 79241-46-6, 69335-90-6	H	5		24
Flucetosulfuron	2				412928-75-7, 412928-69-9	H			
Flumipropyn	1				84478-52-4	H			
Flurochloridone	2		Fluorochloridone		61213-25-0	H			
Fluroxypyr[d]	1 each			***-2-Butoxy-1-methylethyl, -meptyl***[d]	69377-81-7, 154486-27-8, 81406-37-3	H			18
Flurprimidol	1				56425-91-3	PGR			
Flurtamone	1				96525-23-4	H			
Fosamine	1			-Ammonium	59682-52-9, 25954-13-6	H			
Furilazole	1				121776-33-8, 121776-57-6	HS			
Furyloxyfen	1				80020-41-3	H			
Gibberellic acid	8 (6)			-Potassium	77-06-5	PGR			
Gibberellins	8 (6) each			-A4, -A7	8030-53-3, 468-44-0, 510-75-8	PGR			

Glufosinate	2		Phosphinothricin, -P	-Ammonium	53369-07-6, 51276-47-2, 35597-44-5, 77182-82-2	H			
Haloxyfop	1		-P	-(P)-Etotyl, -(P)-methyl, -sodium	69806-34-4, 87237-48-7, 95905-78-5, 95977-29-0, 72619-32-0, 69806-40-2, 69806-86-6	H	5		4
Heptopargil	2 (2)				73886-28-9	PGR			
Imazamethabenz	1 each			-Methyl	100728-84-5, 81405-85-8	H	2		3
Imazamox	1			-Ammonium	114311-32-9, 247057-22-3	H			18
Imazapic	1		AC 263,222	-Ammonium, imazameth	104098-48-8, 104098-49-9	H			13
Imazapyr	1			-Isopropylammonium	81334-34-1, 81510-83-0	H	1		15
Imazaquin	1			-Ammonium, -methyl, -sodium	81335-37-7, 81335-47-9, 81335-43-5, 81335-46-8	H	2		18
Imazethapyr	1			-Ammonium	81335-77-5, 101917-66-2	H			18
Inabenfide	1				82211-24-3	PGR			
Indanofan	1				133220-30-1	H			1
Indaziflam	3				950782-86-2	H			
Isonoruron	5 (4) each	Y			28805-78-9	H			
Isopyrimol	1				55283-69-7	PGR			
Isoxapyrifop	1				87757-18-4	H			
Jasmonic acid	2				6894-38-8	PGR		1	28
Lactofen	1				77501-63-4	H			36

(continued)

Table 3 (continued)

Herbicide name	Chiral features (constrained)	*Meso*?	Alternate names, isomers	Salts, esters	CAS® #s	Type	CE	GC	LC
Mecoprop	1		MCPP, -P	-(P)-Butotyl, -sodium, -(P)-dimethylammonium, -(P)-potassium, -diolamine, -ethadyl, -isoctyl, -methyl, -trolamine, -P-isobutyl	7085-19-0, 23359-62-8, 19095-88-6, 16484-77-8, 66423-09-4, 66423-05-0, 32351-70-5, 1432-14-0, 28473-03-2, 2786-19-8, 1929-86-8, 53404-61-8, 101012-85-5	H	15	7	44
Mefenpyr	1			-Diethyl	135591-00-3, 135590-91-9	HS			
Metamifop	1				256412-89-2	H			
Metobenzuron	1				111578-32-6	H			
Metolachlor	2		*S*-Metolachlor		51218-45-2, 87392-12-9, 178961-20-1	H	3	8	13
Morfamquat	4	Y		-Dichloride	7411-47-4, 4636-83-3	H			
N-Acetylthiazolidine-4-carboxylic acid	1				5025-82-1	PGR			
Naproanilide	1				52570-16-8	H	1		5
Napropamide	1				15299-99-7, 41643-35-0, 41643-36-1	H			18
NC-330	1				Not listed	H			
Noruron	5 (4)		Norea		18530-56-8, 2163-79-3	H			
n-Propyl dihydrojasmonate	2		Prohydrojasmon, PDJ		158474-72-7	PGR			
Paclobutrazol	2				76738-62-0	PGR	2		44
Pentanochlor	1		Solan, CMMP, *N*-(3-chloro-4-*m*ethylphenyl)-2-*m*ethyl*p*entanamide		2307-68-8	H			

Piperophos	1				24151-93-7	H	
Piproctanyl	1			-Bromide	69309-47-3, 56717-11-4	PGR	
Profluazol	2 (1)				190314-43-3	H	
Profoxydim	3		Clefoxydim		139001-49-3	H	
Propisochlor	1		2-Chloro-6′-ethyl-*N*-isopropoxymethylaceto-*o*-toluidide		86763-47-5	H	
Prynachlor	1				21267-72-1	H	
Pydanon	1				22571-07-9	PGR	
Pyriftalid	1				135186-78-6	H	
Pyrimisulfan	1				221205-90-9	H	
Quizalofop	1		Propaquizafop, assure, -P	-Ethyl, -P-ethyl, -P-tefuryl	76578-12-6, 76578-14-8, 94051-08-8, 100646-51-3, 119738-06-6, 111479-05-1	H	49
Sebuthylazine	1				7286-69-3	H	
Secbumeton	1				26259-45-0	H	
Sethoxydim	2				74051-80-2, 71441-80-0	H	1
Siduron	2				1982-49-6	H	
Tefuryltrione	1				473278-76-1	H	
Tepraloxydim	1				149979-41-9	H	
Tetcyclacis	6 (6)				77788-21-7	PGR	
Tiocarbazil	2	Y			36756-79-3	H	
Tralkoxydim	1				87820-88-0	H	
Triapenthenol	1				76608-88-3	PGR	1
Triaziflam	1				131475-57-5	H	
Tridiphane	1				58138-08-2	H	

(continued)

Table 3 (continued)

Herbicide name	Chiral features (constrained)	*Meso*?	Alternate names, isomers	Salts, esters	CAS® #s	Type	CE	GC	LC
Trifop	1			-Methyl	58594-74-4, 59011-30-2, 58594-77-7, 59011-33-5	H			
Trifopsime	1				72131-76-1	H			
Tritac	1				1861-44-5	H			
UBI-S734	1		Dimenoxypyrin		60263-88-9	H			
Uniconazole	1		-P		83657-22-1, 83657-17-4, 83657-16-3, 76714-83-5	PGR	2		64

[a]The parent herbicide 2,4-D is achiral, however, the *-2 -butoxypropyl* (1 chiral feature, cf), *-2-ethylhexyl* (1 cf), *lvxiancaolin* (1 cf), *-meptyl* (1 cf), *-tefuryl* (1 cf), *triisopropanolammonium* (3 cf, *meso* compound) esters and salts are chiral. The salt counter ions and ester moiety are not likely to remain with the 2,4-D parent portion indefinitely. However, these salts and esters have been included as examples of the potential for chirality by esters and salts within an achiral pesticide component, and for completeness

[b]-Ammonium [2307-55-3], -butotyl [1929-73-3], -2-butoxypropyl [1320-18-9], -3-butoxypropyl [1928-45-6], -butyl [94-80-4], -diethylammonium [20940-37-8], -dimethylammonium [2008-39-1], -diolamine [5742-19-8], -dodecylammonium [2212-54-6], -ethyl [533-23-3], -2-ethylhexyl [1928-43-4], -heptylammonium [37102-63-9], -isobutyl [1713-15-1], -isoctyl [25168-26-7], -isopropyl [94-11-1], -isopropylammonium [5742-17-6], -lvxiancaolin [215655-76-8], -lithium [3766-27-6], -meptyl [1917-97-1], -methyl [1928-38-7], -octyl [1928-44-5], -pentyl [1917-92-6], -propyl [1928-61-6], -sodium [2702-72-9], -tefuryl [15146-99-3], -tetradecylammonium [28685-18-9], -triethylammonium [2646-78-8], -triisopropanolammonium [18584-79-7, 32341-80-3], -trolamine [2569-01-9]

[c] Names listed in bold refer to the chiral form. The parent herbicide cloquintocet is achiral, however, the **-mexyl** ester is chiral

[d]The parent herbicide fluroxypyr is achiral, however, the **-2-butoxy-1-methylethyl** and **-meptyl** esters are chiral

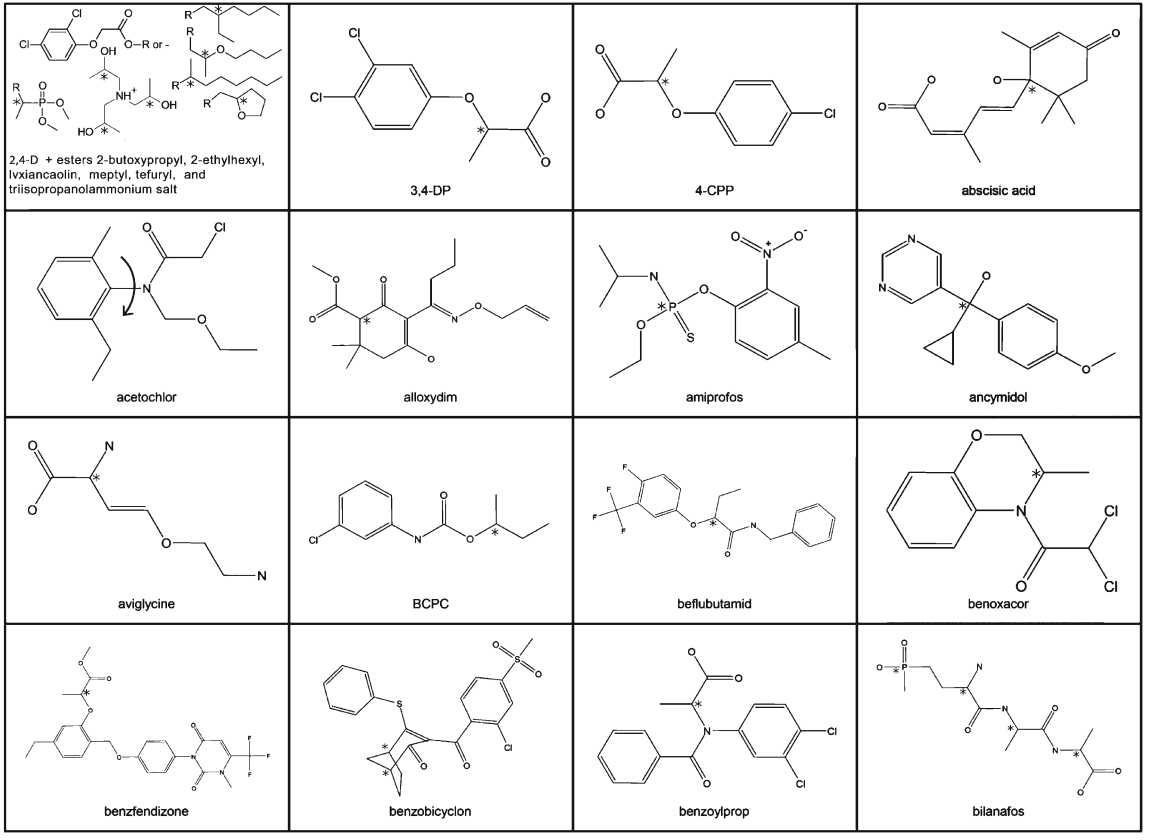


Fig. 4 Chiral herbicide, herbicide safener, and plant growth regulator structures. See Fig. 2 for symbol description

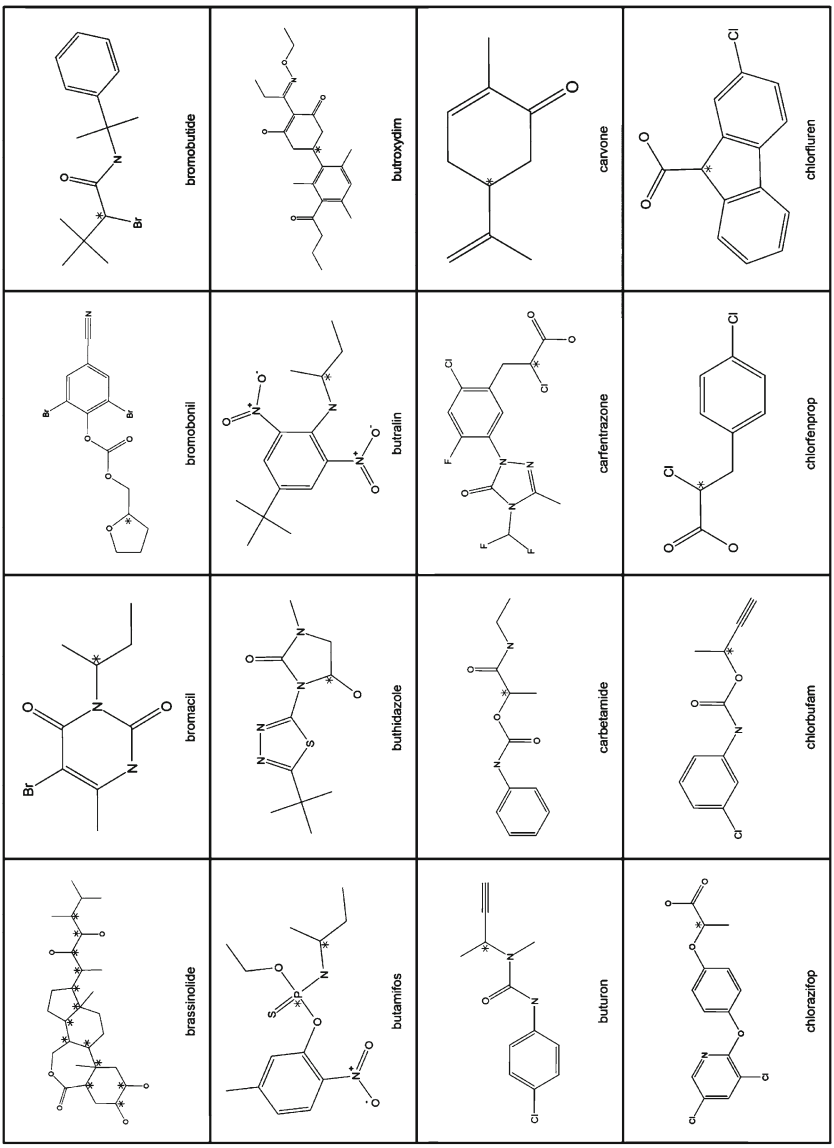


Fig. 4 (continued)

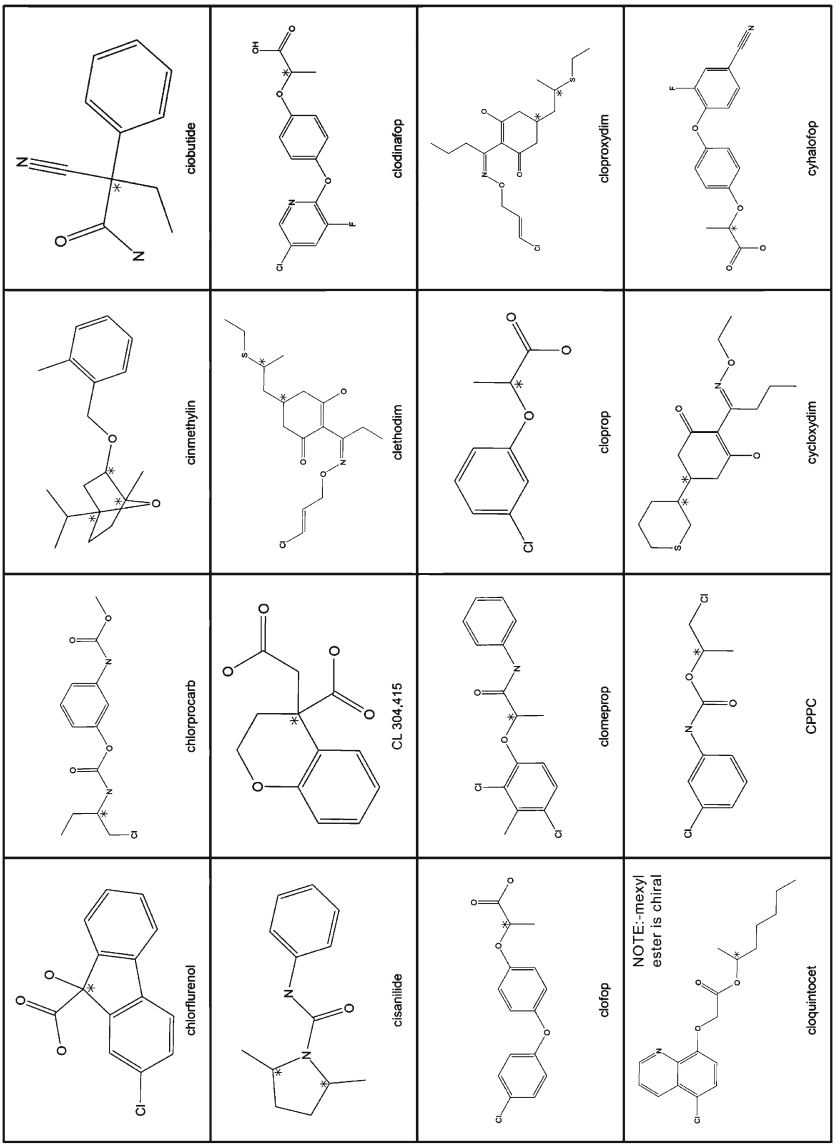


Fig. 4 (continued)

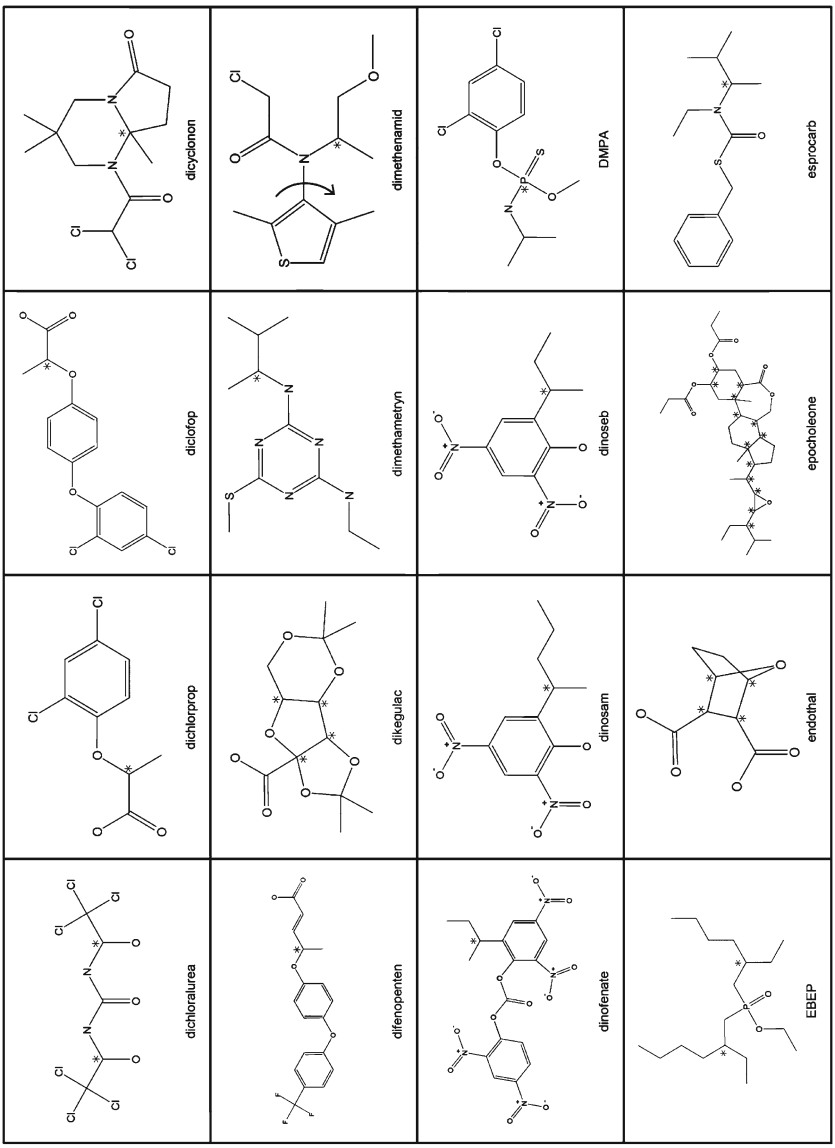


Fig. 4 (continued)

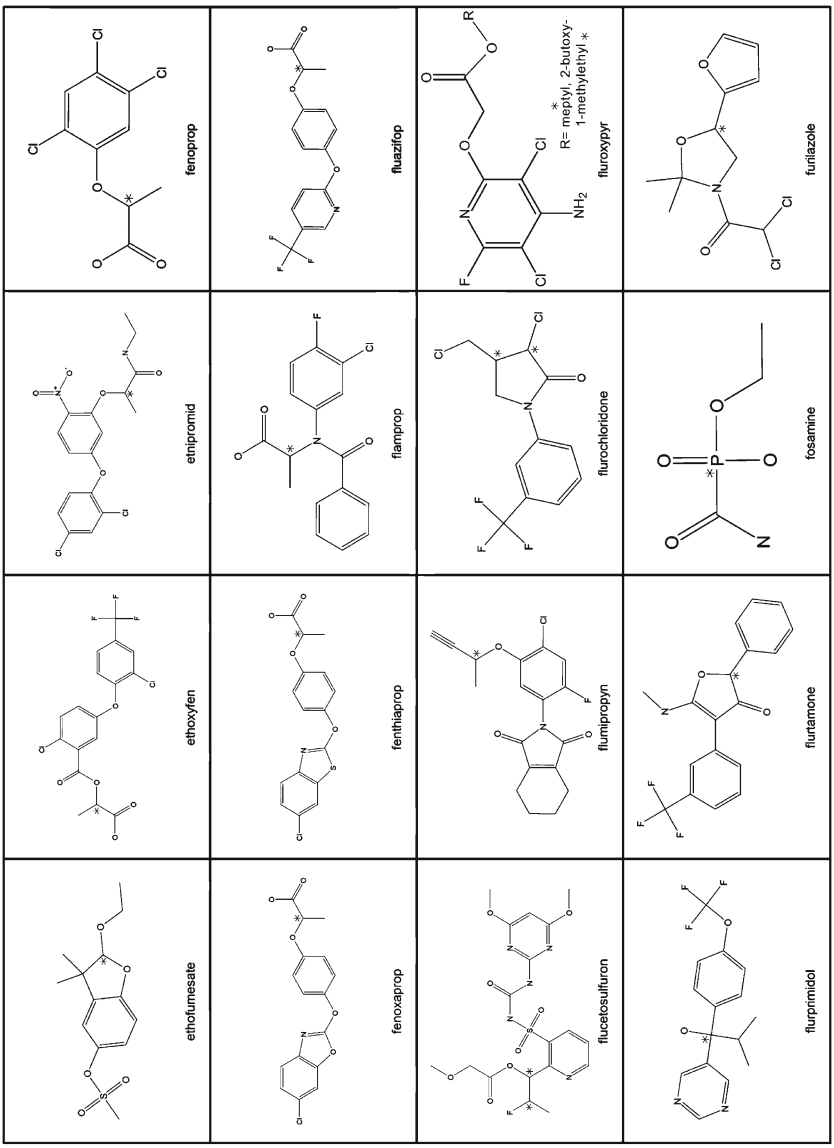


Fig. 4 (continued)

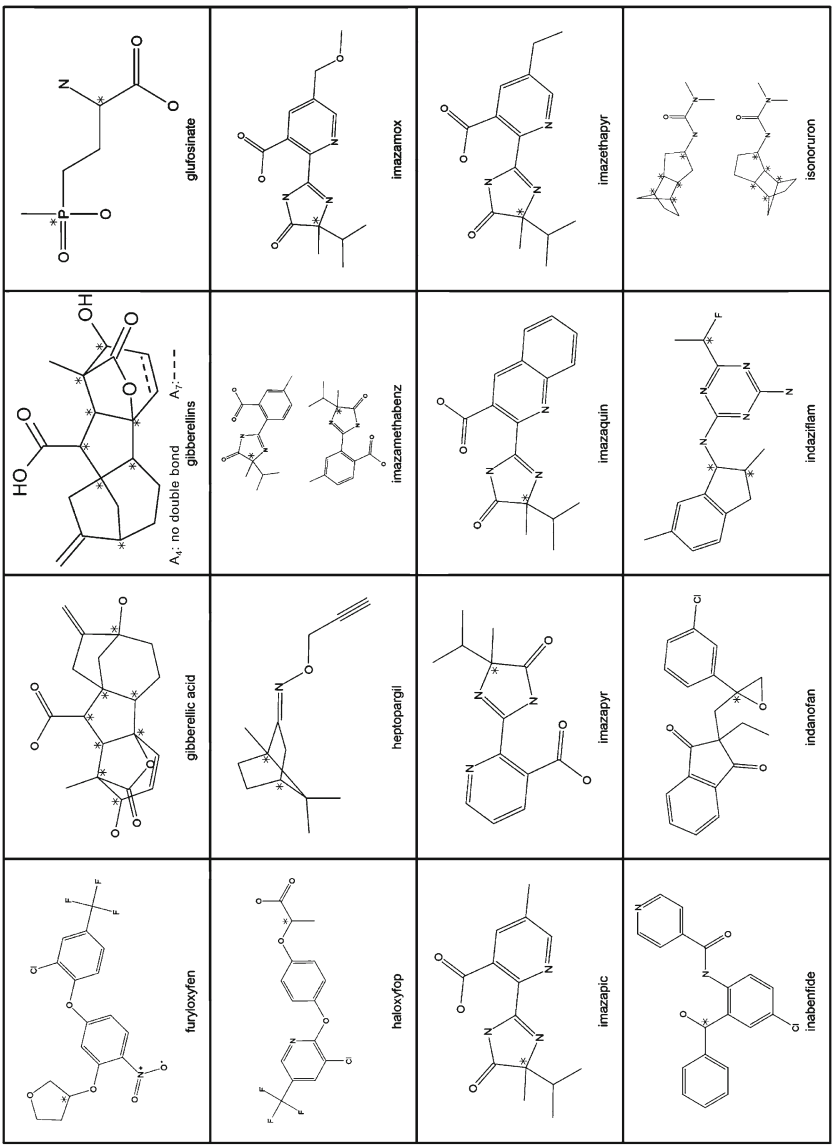


Fig. 4 (continued)

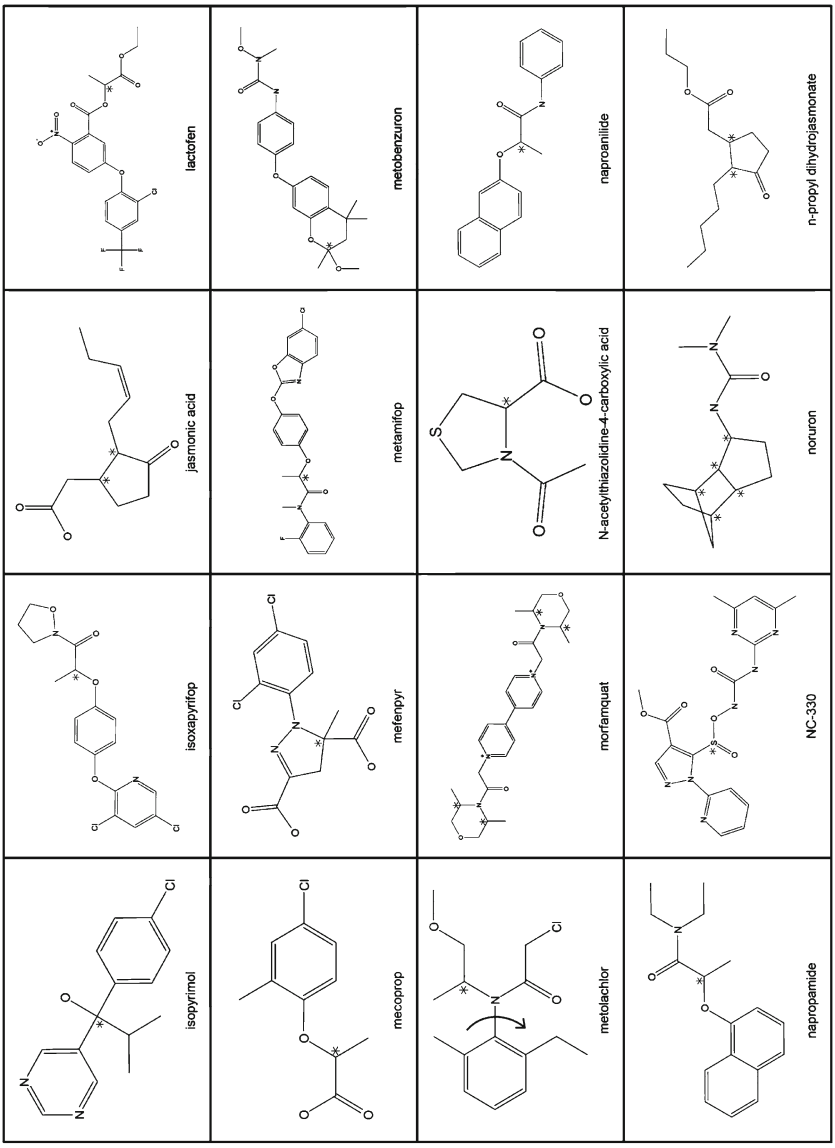


Fig. 4 (continued)

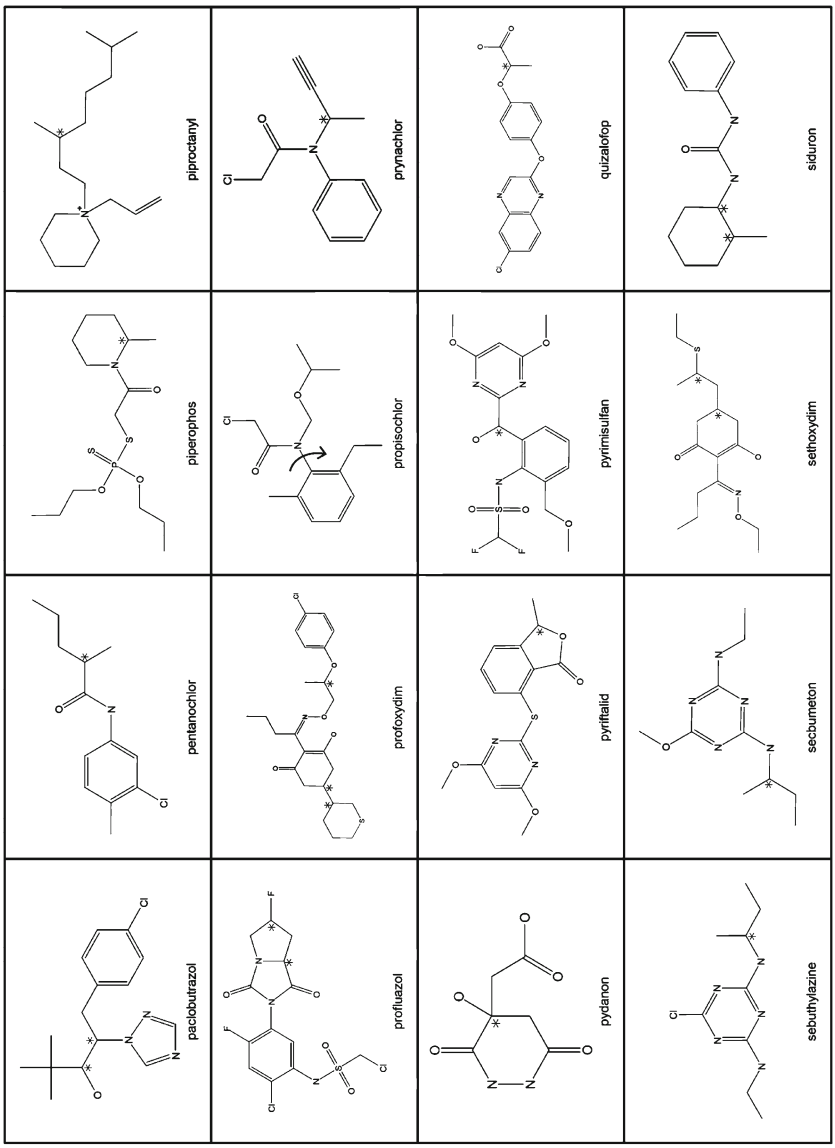
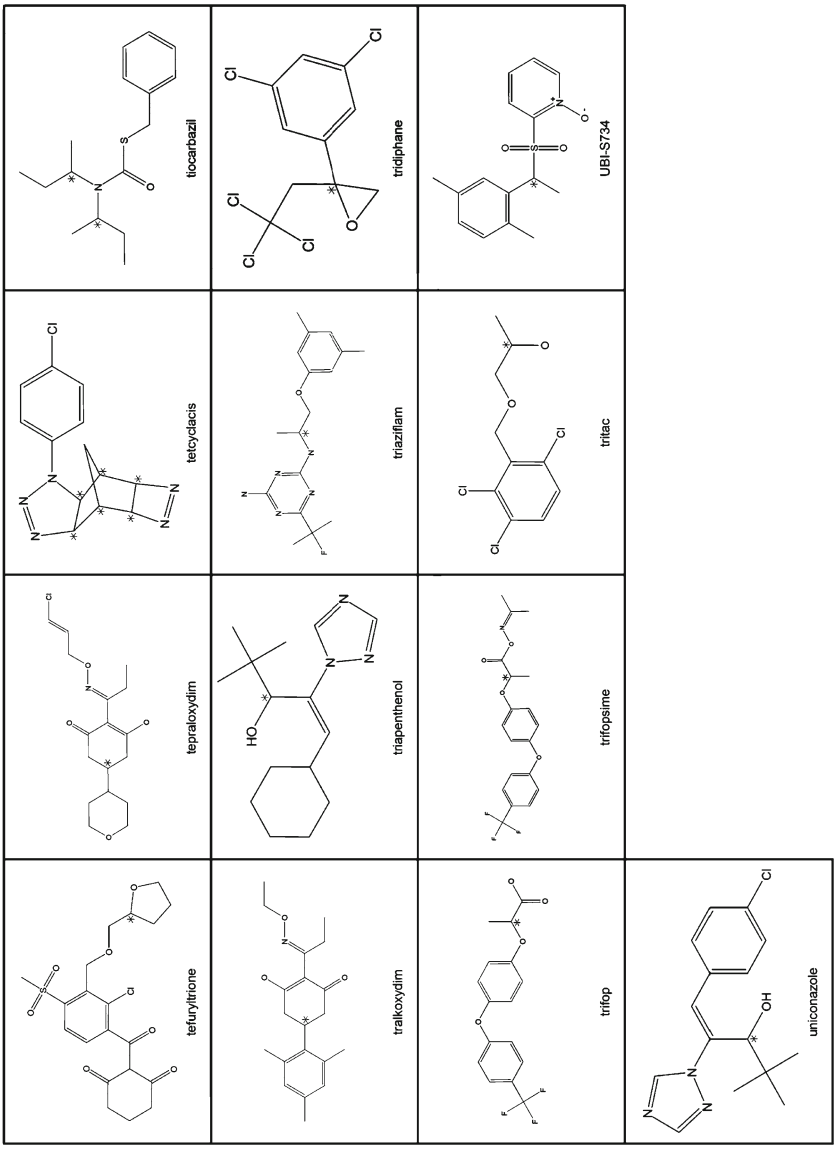


Fig. 4 (continued)


tiocarbazil
tridiphane
UBI-S734
tetcyclacis
triaziflam
tritac
tepraloxydim
triapenthenol
trifopsime
tefuryltrione
tralkoxydim
trifop
uniconazole

We have included the herbicide 2,4-D [(*2,4-d*ichlorophenoxy)acetic acid] in Table 3 and Fig. 4 as an example of an acid herbicide that can be paired with chiral esters or salts, but the herbicide itself is not chiral. Any achiral acid herbicide that is paired with such chiral moieties will be chiral as long as the bonding is sustained. The pairings, especially the salt forms, may be short-lived following application due to rapid dissociation of the salt moiety or degradation via abiotic and biotic pathways (e.g., ester hydrolysis). For clarity, please see footnotes in the tables for such compounds. The majority of acid herbicides and other acid functional pesticides covered in this review contain a parent group moiety that is chiral [e.g., 3,4-DP; 2-(*3,4-d*ichlorophenoxy)*p*ropanoic acid], but these pesticides may contain additional chiral centers (and thus stereoisomers) that depend on the formulation. We have noted some of the more common ester and salt forms in the tables, but these listings are not comprehensive.

The mode of action of many herbicides is to interfere with chiral plant hormones controlling growth, so it is not surprising that the absolute configuration of the pesticide plays a role in efficacy (Naber and van Rensen 1988). The degradation of dichlorprop and mecoprop by soil microbes is enantioselective because two different enzymes each metabolize one enantiomer (Zipper et al. 1996; Nickel et al. 1997; Kohler et al. 1998; Zipper et al. 1998; Müller and Babel 1999). These two examples demonstrate how the degradation is different between stereoisomers, and this, when combined with stereospecific toxicity, can affect not only efficacy, but also exposure and risk to humans and other nontarget organisms in the environment.

ChirBase contains entries for 44 of the 141 herbicides (31%). There are 972 records for these 44 pesticides (766 in LC, 120 in GC, and 86 in CE databases). Carvone has the most entries, with a total of 91, of which 76 are found in the GC database. The herbicides found in ChirBase are shown in Table 3. The four stereoisomers of paclobutrazol were separated by GC (Clark and Deas 1985), and the enantiomers of cloprop were separated by CE (Tang et al. 2005). Neither of these example herbicides is found as entries in ChirBase.

4.3 Insecticides

In Table 4, we list 149 chiral insecticides, and show their structures in Fig. 5. Thirty-three insect attractants, pheromones, repellents, and insecticide synergists were included in the miscellaneous category and are discussed in the next section. The majority of the insecticides have one to four chiral features, but several biologically derived insecticides (e.g., abamectin, allosamidin, azadirachtin, emmamectin, sabadilla, spinetoram, and spinosad) have more than a dozen chiral features. Twenty-seven insecticides are chiral at a phosphorus atom (Fig. 5), which is not surprising considering that numerous organophosphorus (OP) insecticides are included in this group. The following compounds all have a chiral sulfur atom in their structure: 2,2-dichlorovinyl 2-ethylsulfinylethyl methyl phosphate, ethiprole, fipronil, IPSP (*S*-ethylsulfinylmethyl *O*,*O*-diisopropyl phosphorodithioate), mesulfenfos,

Table 4 Chiral insecticides

Insecticide name	Chiral features (constrained)	*Meso*?	Alternate names, isomers[a]	Salts, esters	CAS® #s	CE	GC	LC
1,1-Dichloro-2,2-bis (4-ethylphenyl)ethane	1		**o,p′-**[a]; perthane, ethylan, ethyl-DDD, *di*chloro*di*phenyl-*di*chloroethane		72-56-0			
1,2-Dichloropropane with 1,3-dichloropropene	1				8003-19-8, 78-87-5, 542-75-6, 10061-02-6, 10061-01-5		6	
2-(4,5-Dimethyl-1,3-dioxolan-2-yl)phenyl methylcarbamate	2	Y			7122-04-5			
2,2,2-Trichloro-1-(3,4-dichlorophenyl) ethyl acetate	1		Plifenate, acetofenate, penfenate		21757-82-4			
2,2-Dichlorovinyl 2-ethyl-sulfinylethyl methyl phosphate	2				7076-53-1			
Abamectin	19/20 (2)		Avermectin B_{1a}, B_{1b}		71751-41-2, 65195-55-3, 65195-56-4			
Acephate	1		Orthene		30560-19-1		1	11
Aldrin	6 (6)	Y	Isodrin, HHDN, 1,2,3,4,10,10-*h*exachloro-1,4,4a,5,8,8a-*h*exahydro-1, 4:5,8-*di*methano*n*aphthalene		309-00-2, 465-73-6			
Allethrin	3		*d*-Allethrin; (*S*)-bioallethrin, *d-trans*-allethrin, -*S*-cyclopentyl isomer, esbiol, esbiothrin, depallethrine, palléthrine		584-79-2, 28434-00-6	1	10	26
Allosamidin	15 (2)				103782-08-7			
Anabasine	1				494-52-0, 13078-04-1	1	1	3

(continued)

Table 4 (continued)

Insecticide name	Chiral features (constrained)	*Meso*?	Alternate names, isomers[a]	Salts, esters	CAS® #s	CE	GC	LC
Azadirachtin	16 (11)		Extract of neem oil		11141-17-6, 8002-65-1			
Barthrin	2				70-43-9			
Bifenthrin	2				82657-04-3	0	0	3
Bioethanomethrin	2				22431-62-5			
Bromocyclen	3 (2)		Bromociclen		1715-40-8	1	5	
Bufencarb	1		**1-Methylbutyl**[a]		8065-36-9, 2282-34-0, 672-04-8			
Butocarboxim	1				34681-10-2			
Butoxycarboxim	1				34681-23-7			
Chlorbicyclen	4 (2)	Y			2550-75-6			
Chlordane	6 (4)				57-74-9, 12789-03-6, 5103-71-9, 5103-74-2		64	11
Chlordecone	4	Y			143-50-0			
Chlorethoxyfos	1				54593-83-8			
Cloethocarb	1				51487-69-5			
Crotoxyphos	1		Crotoxyfos		7700-17-6			3
Crufomate	1				299-86-5			
CS 708	1				117-26-0, 117-27-1, 8027-00-7			7
Cyanofenphos	1				13067-93-1			
Cyclethrin	4				97-11-0			
Cycloprothrin	2				63935-38-6			
Cyfluthrin	3		Beta-		68359-37-5, 86560-92-1, 86560-93-2, 86560-94-3, 86560-95-4			20
Cyhalothrin	3		Karate, gamma-, lambda-		68085-85-8, 76703-62-3, 91465-08-6			13

Cypermethrin	3		Alpha-, beta-, theta-, zeta-		52315-07-8, 67375-30-8, 86753-92-6, 72204-43-4, 65731-84-2, 83860-31-5, 65732-07-2, 71697-59-1,	4	1	76
Cyphenothrin	3		*d*-, (1*R*)-*trans*- isomers		39515-40-7			
DDD	1		TDE, **o,p′-**, 1,1-dichloro-2, 2-bis(4-chlorophenyl)ethane		72-54-8	1	7	9
DDT	1		*p,p′*-, **o,p′-**, clofenotane, *d*ichloro*d*iphenyl*t*richloroethane		50-29-3, 8017-34-3	1	11	14
Decarbofuran	1				1563-67-3			
Deltamethrin	3		Decamethrin		52918-63-5, 52820-00-5			12
Dialifos	1		Dialifor, torak		10311-84-9	1		
Dieldrin	8 (8)	Y	Endrin, nendrin, HEOD, 1,2,3,4,10,10-*h*exachloro-6, 7-*e*poxy-1,4,4a,5,6,7,8, 8a-*o*ctahydro-endo-1,4-exo-5, 8-*d*imethanonaphthalene		60-57-1, 72-20-8			
Dilor	4 (4)	Y	Chlordane compound		14168-01-5			
Dimefluthrin	2				271241-14-6			
Dimethrin	2				70-38-2			
Dinotefuran	1				165252-70-0			
Diofenolan	2				63837-33-2			
Dioxabenzofos	1		Salithion		3811-49-2			
Dioxathion	2	Y	Dioxation, delnav		78-34-2			
Dithicrofos	1				41219-31-2			
d-Limonene	1				5989-27-5	1	168	6
Ecdysone	10 (5)		α-, β-, ecdysterone, hydroxyexdysone		3604-87-3, 5289-74-7			
Emamectin	19/20 (2)			-Benzoate	119791-41-2, 155569-91-8			

(continued)

Table 4 (continued)

Insecticide name	Chiral features (constrained)	*Meso*?	Alternate names, isomers[a]	Salts, esters	CAS® #s	CE	GC	LC
Empenthrin	3				54406-48-3			
Endosulfan	4 (4)	Y	Alpha-, beta-, thiodan, benzoepin		115-29-7, 959-98-8, 33213-65-9		2	1
EPBP	1		*O-E*thyl *O*-2,4-dichloro*p*henyl thiono*b*enzene*p*hosphonate		3792-59-4			
EPN	1		*O-E*thyl *O-p-n*itrophenyl phenylphosphonothioate		2104-64-5			1
Epofenonane	3				57342-02-6			
Etaphos	1				38527-91-2			
Ethiprole	1				181587-01-9			
Fenfluthrin	2				75867-00-4			
Fenobucarb	1				3766-81-2			1
Fenpirithrin	3				68523-18-2			
Fenvalerate	2		Esfenvalerate, fenvalerate-U		66230-04-4, 51630-58-1			31
Fipronil	1				120068-37-3			39
Flucythrinate	2				70124-77-5			1
Flufenerim	1				170015-32-4			
Flufenprox	1				107713-58-6			
Flumethrin	3				69770-45-2			1
Fluvalinate	2		Tau-		69409-94-5, 102851-06-9			10
Fonofos	1				944-22-9, 66767-39-3, 62705-71-9, 62680-03-9		1	10
Furethrin	3				17080-02-3			
HCH	1		**Alpha**-[a], beta-, gamma-lindane, delta-, eta-, theta-, zeta-, epsilon-, *h*exa*c*hlorocyclo-*h*exane, benzene hexachloride		608-73-1, 319-84-6, 319-85-7, 58-89-9, 319-86-8, 6108-10-7		2	2

Heptachlor	5 (4)				76-44-8		47	6
Heptenophos	2 (2)				23560-59-0			
Heterophos	1				40626-35-5			
Hydroprene	1		(S)-		41205-09-8, 41096-46-2, 65733-18-8, 65733-19-9			
Imiprothrin	2				72963-72-5			
Indoxacarb	1				144171-61-9, 173584-44-6			
IPSP	1		*S*-Ethylsulfinylmethyl *O*,*O*-diisopropyl phosphorodithioate		5827-05-4			
Isobenzan	6 (4)	Y			297-78-9			
Isofenphos	1			-Methyl	99675-03-3, 25311-71-1			12
Isopropyl *O*-(methoxyamino-thiophosphoryl)salicylate	1		Isocarbophos, isocarbofos		24353-61-5			
Juvenile hormone I	2				13804-51-8			1
Juvenile hormone II	2				34218-61-6			1
Juvenile hormone III	1				22963-93-5	1		1
Kelevan	4 (Y)	Y			4234-79-1			
Kinoprene	1		(*S*)-		42588-37-4, 65733-20-2			
Lepimectin	11 (2)				171249-05-1, 171249-10-8			
Leptophos	1				21609-90-5			3
Lufenuron	1				103055-07-8			1
Malathion	1		Carbophos, maldison, mercaptothion		121-75-5			41
Mecarphon	1				29173-31-7			
Mephosfolan	1				950-10-7			
Mesulfenfos	1				3761-41-9			
Methamidophos	1				10265-92-6		2	23
Methoprene	1		(*S*)-		40596-69-8, 41205-06-5, 65733-16-6, 65733-17-7			

(continued)

Table 4 (continued)

Insecticide name	Chiral features (constrained)	*Meso*?	Alternate names, isomers[a]	Salts, esters	CAS® #s	CE	GC	LC
Methothrin	2				34388-29-9, 11114-02-6			
Methoxychlor	1		**o,p'**-[a]		72-43-5, 30667-99-3			
Metofluthrin	2				240494-70-6			
Naled	1		Dibrom, BRP *br*omchlo*p*hos,		300-76-5			
Nicotine	1			-Sulfate	54-11-5, 22083-74-5, 75202-10-7, 65-30-5	1	3	34
Nornicotine	1				494-97-3	1	11	41
Novaluron	1				116714-46-6			
Noviflumuron	1				121451-02-3			
O-2,5-Dichloro-4-iodophenyl *O*-ethyl ethylphosphonothioate	1				25177-27-9			
Oxydemeton-methyl	1		Methylmercaptophos oxide		301-12-2			1
Oxydeprofos	2		ESP, *S*-[2-(*e*thyl*s*ulfinyl)-1-methylethyl] *O*,*O*-dimethyl *p*hosphorothioate		2674-91-1			
Oxydisulfoton	1				2497-07-6			
Permethrin	2		*cis*-, *trans*-, bio-		52645-53-1, 61949-77-7, 51877-74-8, 54774-47-9, 61949-76-6, 54774-45-7, 54774-46-8			47
Phenothrin	2		*cis*-, *trans*-, *d*-		26046-85-5, 51186-88-0, 26002-80-2	2		10
Phenthoate	1		PAP		2597-03-7			49
Pirimetaphos	1				31377-69-2			
Prallethrin	3				23031-36-9			1
Profenofos	1				41198-08-7			22

Profluthrin	2		223419-20-3	
Propetamphos	1		31218-83-4	
Prothiofos	1		34643-46-4	
Protrifenbute	1		119544-94-4	
Pyraclofos	1		89784-60-1, 77458-01-6	
Pyresmethrin	2		24624-58-6	
Pyrethrins	3 each	Cinerin, jasmolin, and pyrethrin -I and -II	25402-06-6, 121-20-0, 4466-14-2, 121-21-1, 121-29-9, 1172-63-0, 8003-34-7	
Pyriproxyfen	1		95737-68-1	
Quassia	7 (6)	Quassin	97676-28-3, 84604-10-4, 68915-32-2.	
Quintiofos	1		1776-83-6	
Resmethrin	2	Cismethrin, bio-	10453-86-8, 35764-59-1, 28434-01-7	11
Rotenone	3 (2)	Derris	83-79-4	34
RU 15525	3	Kadethrin	58769-20-3	
RU 25475	4	Tralocythrin	66841-26-7	
Ryanodine	11 (7)	Ryania	15662-33-6, 94513-55-0, 8047-13-0	
Sabadilla	14 (10) each	Cevadine, veratridine, cevadilla	8051-02-3, 62-59-9, 71-62-5	
Spinetoram	17 (5) each		187166-40-1, 187166-15-0	
Spinosad	17 (5) each	Spinosyn -A, -D	168316-95-8, 131929-60-7, 131929-63-0	
Spirotetramat	2		203313-25-1	
Sulfoxaflor	2		946578-00-3	
Sulprofos	1		35400-43-2	

(continued)

Table 4 (continued)

Insecticide name	Chiral features (constrained)	*Meso*?	Alternate names, isomers[a]	Salts, esters	CAS® #s	CE	GC	LC
Tebupirimfos	1				96182-53-5			
Tefluthrin	2				79538-32-2			
Terallethrin	1				15589-31-8			40
Tetramethrin	2		(1*R*)-Isomers, phthalthrin		7696-12-0, 51348-90-4, 1166-46-7			7
Thicrofos	1				41219-32-3			
Toxaphene	Varies (2)[b]		Camphechlor, polychlorcamphene		8001-35-2			
Tralomethrin	4				66841-25-6			
Transfluthrin	2				118712-89-3			
Trichlorfon	1		Metrifonate, DEP, chlorophos, dipterex, metriphonate, trichlorphon, butylchlorophos	-butyrate	52-68-6, 126-22-7		2	1
Trichlormetaphos-3	1				2633-54-7			
Trichloronat	1		Trichloronate		327-98-0			8
Triprene	1		Altorick		40596-80-3			
Vamidothion	1				2275-23-2			
Zolaprofos	1				63771-69-7			
ZXI 8901	2				160791-64-0			

[a]Isomers listed in bold refer to the chiral form

[b]Complex reaction mixture with many chlorination patterns; most congeners contain at least one chiral feature

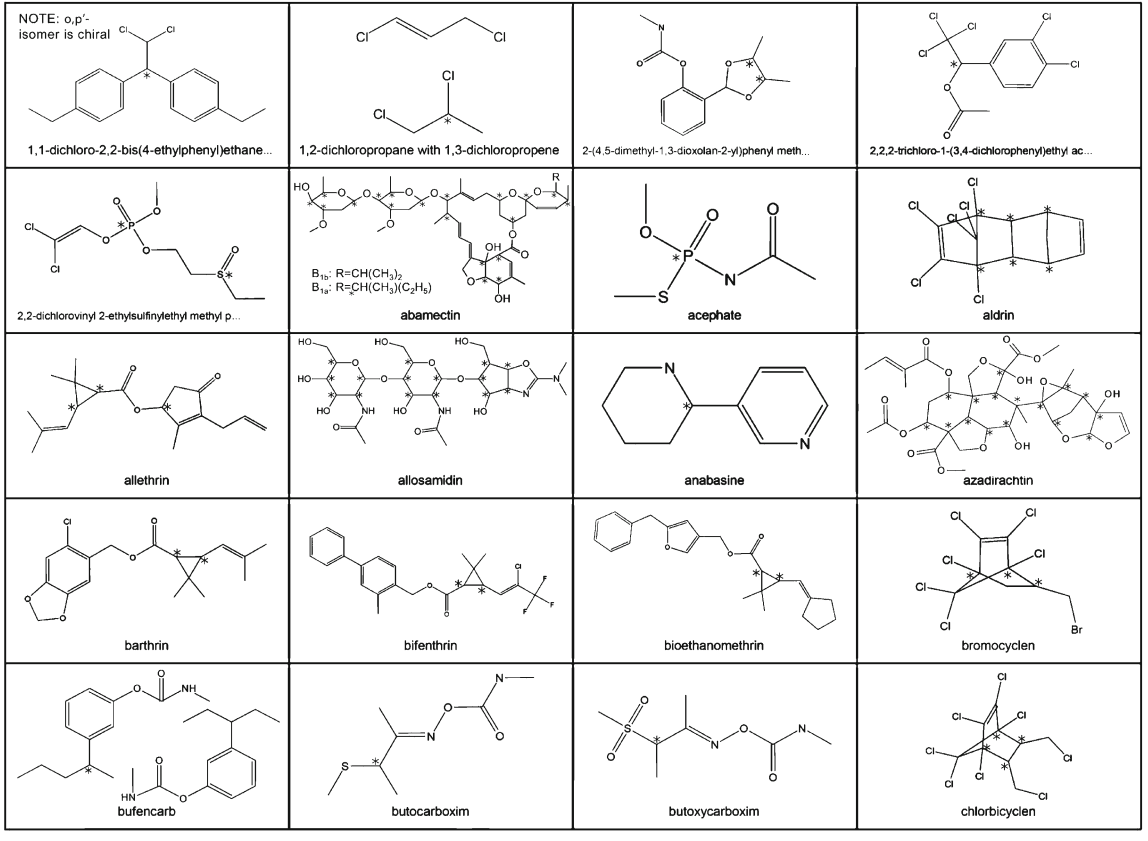


Fig. 5 Chiral insecticide structures. See Fig. 2 for symbol description

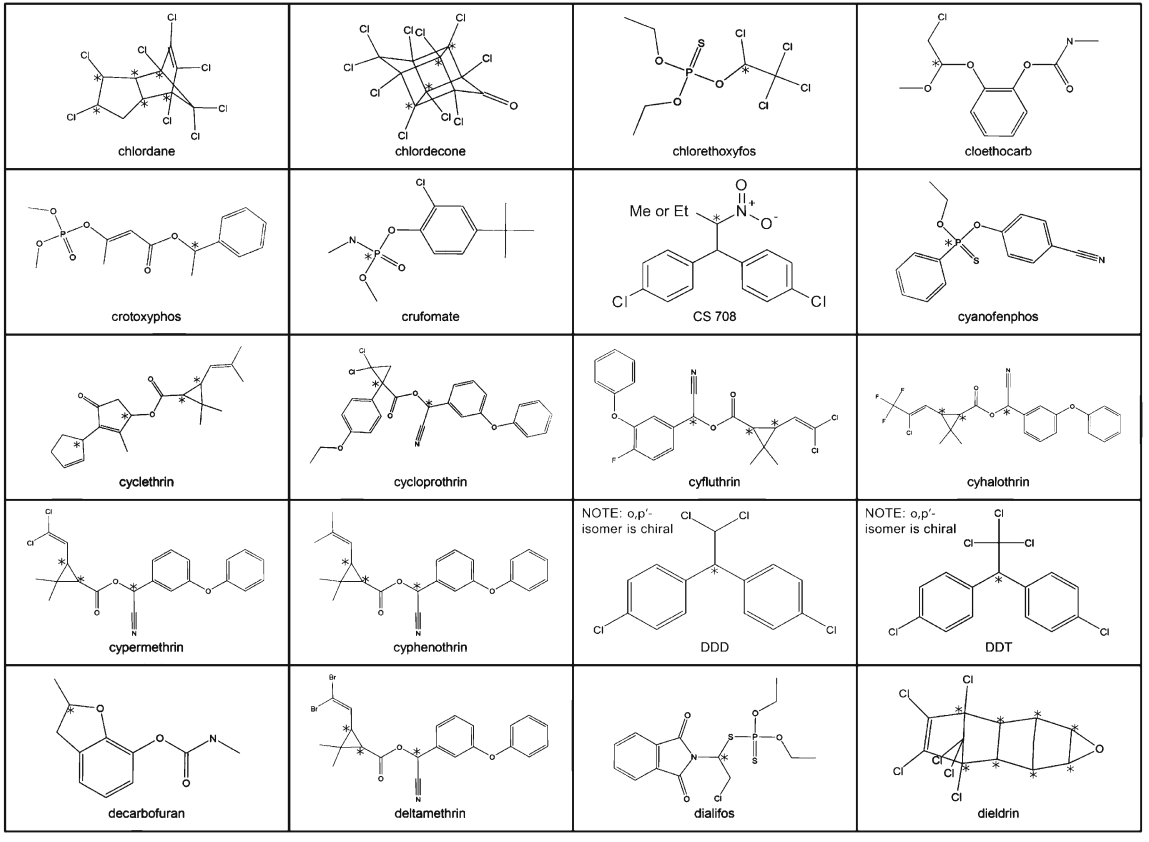
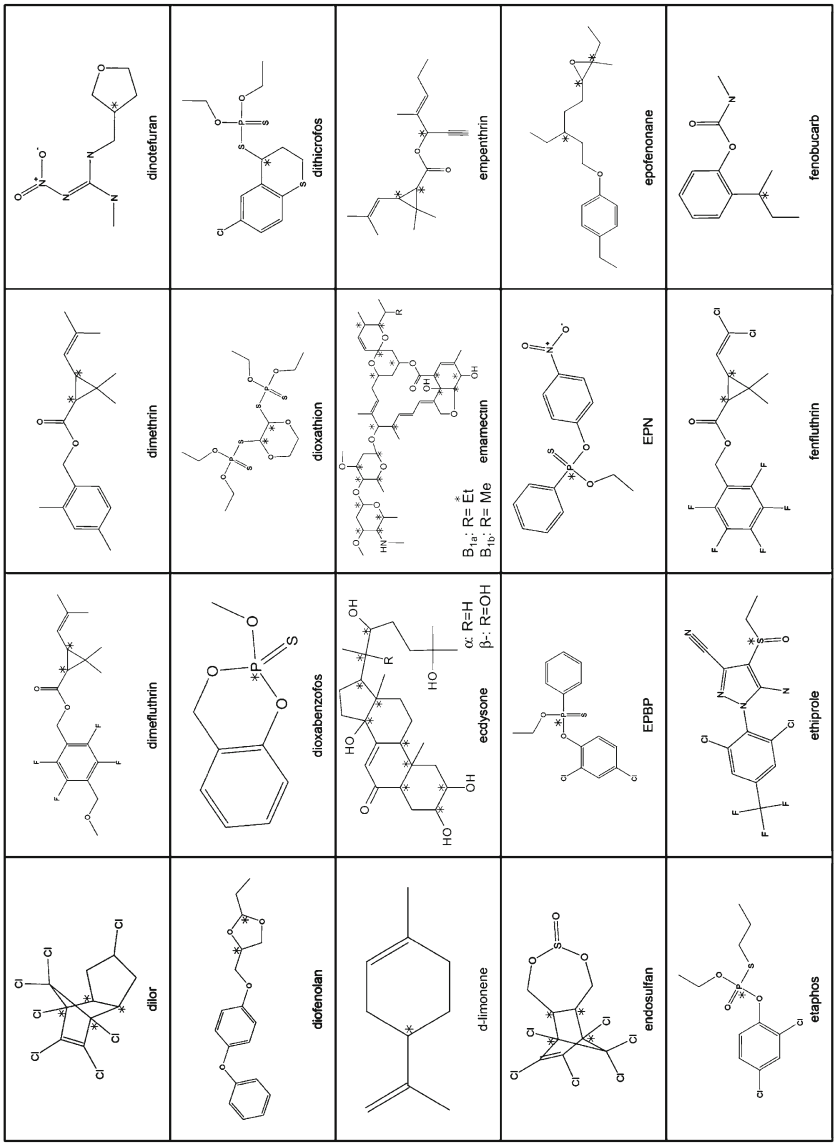


Fig. 5 (continued)



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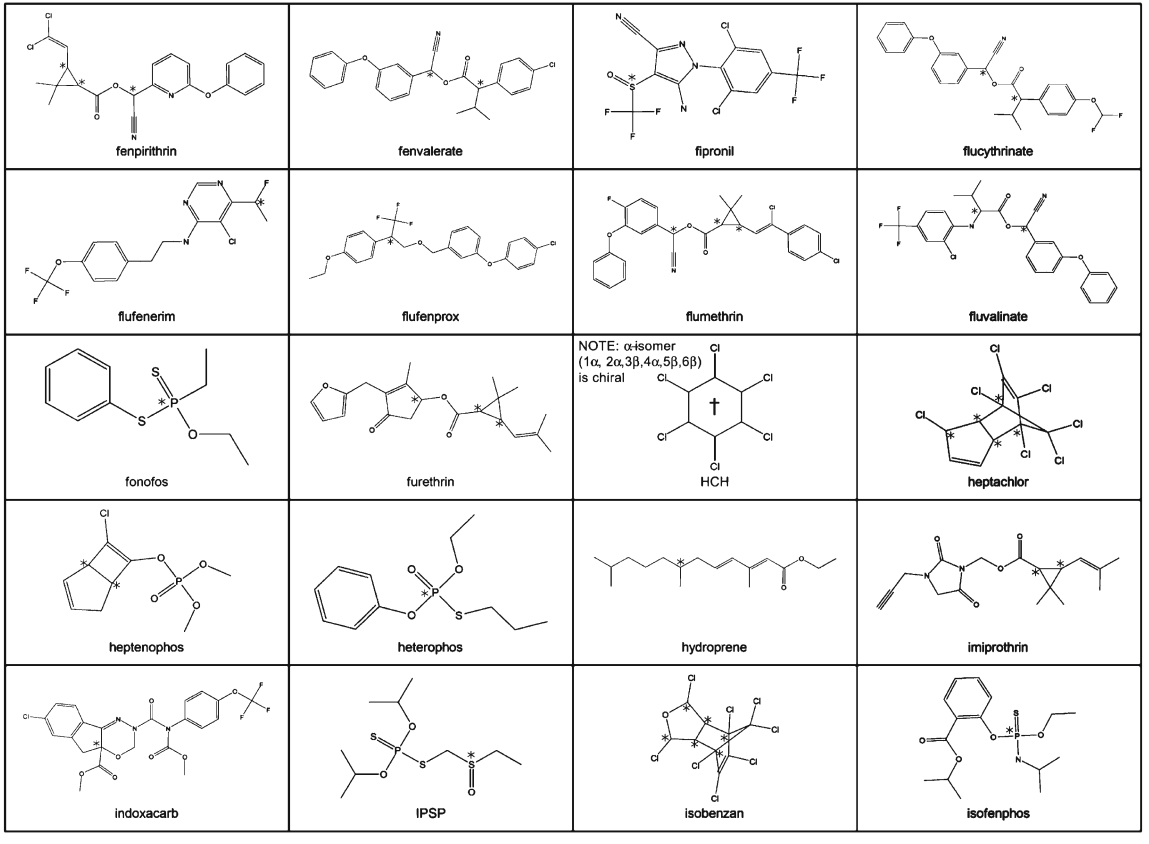

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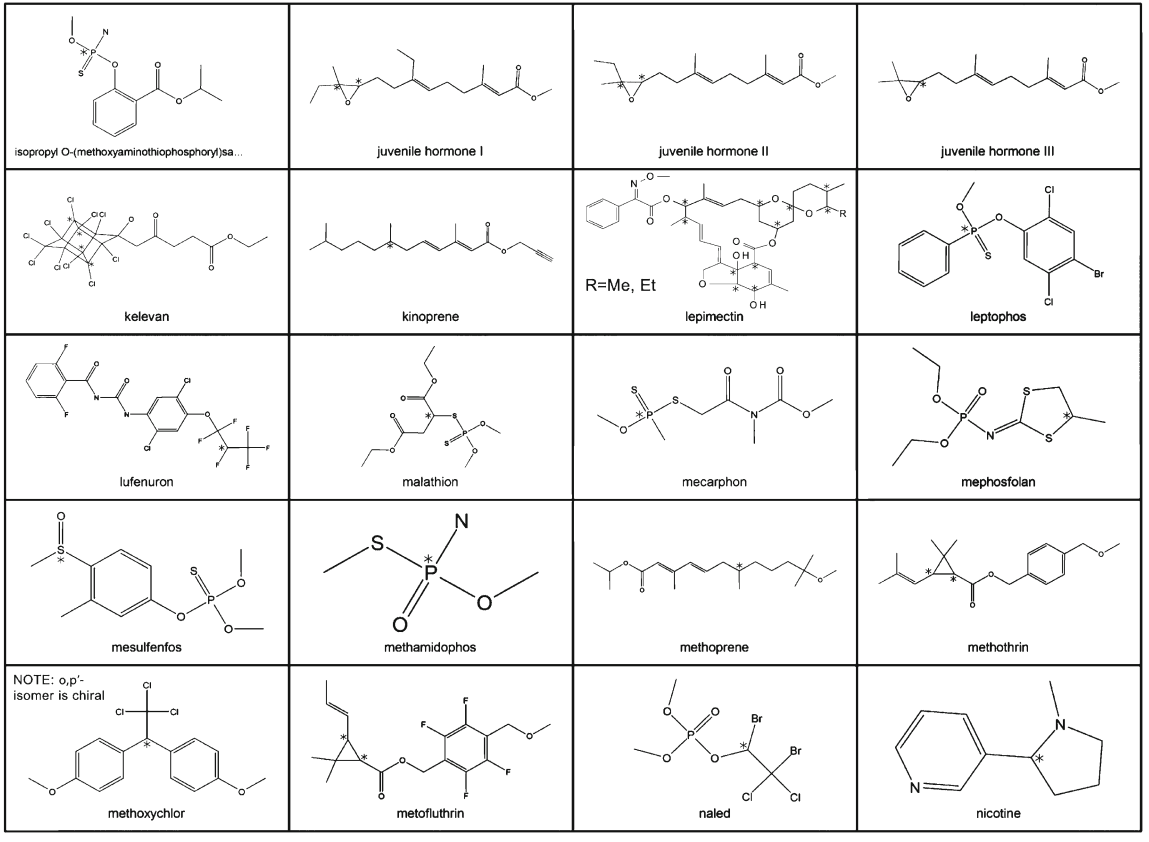

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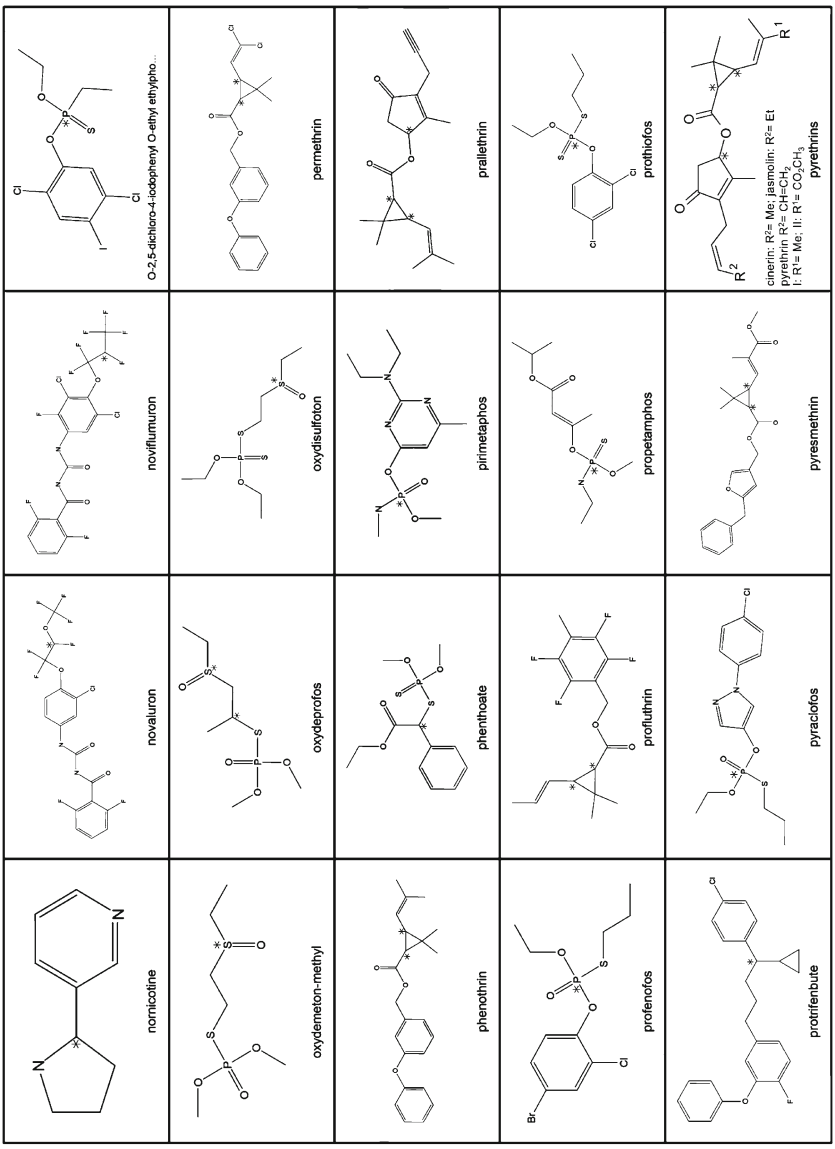

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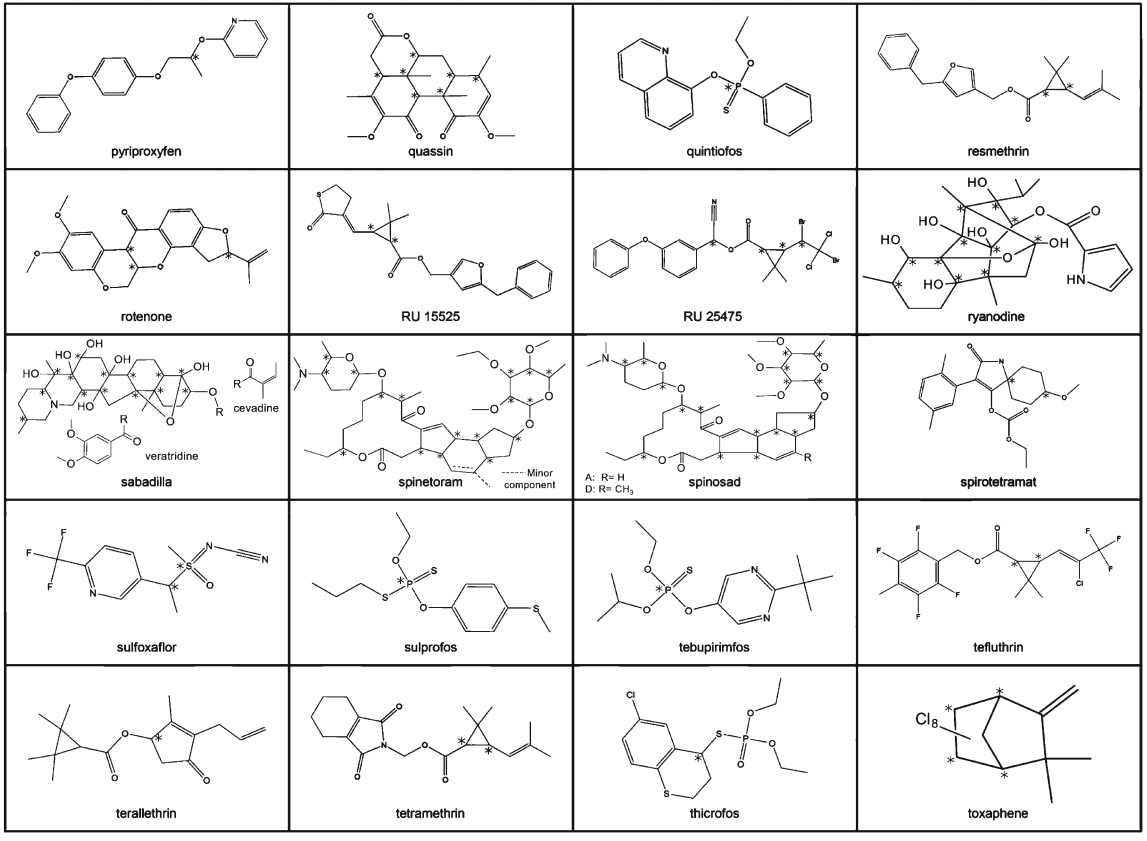
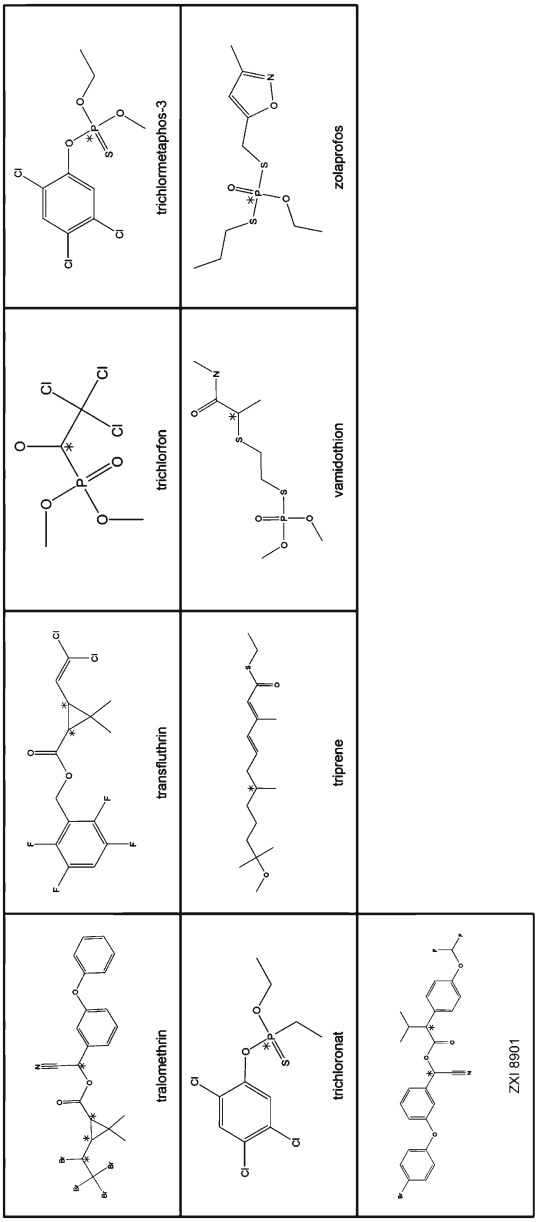


Fig. 5 (continued)


trichlormetaphos-3
zolaprofos
trichlorfon
vamidothion
transfluthrin
triprene
tralomethrin
trichloronat
ZXI 8901

oxydemeton-methyl, oxydeprofos, oxydisulfoton, and sulfoxaflor. α-Hexachlorocyclohexane (HCH) is an example of a molecule that does not contain a point or plane of symmetry, thus it is chiral and has two enantiomers (see Fig. 2d). The other six isomers of HCH, including the active insecticidal form, γ-HCH, or lindane, are achiral (Willett et al. 1998).

There are many chiral insecticides that have ring-constrained chiral features, which limit the actual number of possible stereoisomers. For example, the organochlorine (OC) insecticide chlordane has six chiral carbon atoms, but only two of them are unconstrained (see Fig. 2f), which leads to four possible stereoisomers, a pair of enantiomers for *cis*-chlordane and a pair for *trans*-chlordane. Constrained and *meso*-compounds are noted in Table 4.

At least 17 of the chiral insecticides are produced as single or enriched stereoisomer formulations, including allethrin, cyfluthrin, cyhalothrin, cypermethrin, cyphenothrin, deltamethrin, *d*-limonene, endosulfan, fenvalerate, fluvalinate, hydroprene, kinoprene, methoprene, permethrin, phenothrin, resmethrin, and tetramethrin. For some of the pyrethroid insecticides, multiple commercial formulations have progressed from racemic mixtures to increased enrichment of the active stereoisomer(s) (Williams 1992). Indeed, deltamethrin was specifically developed as a single stereoisomer formulation (Carle et al. 1982).

Organochlorine pesticides are among the most widely studied classes of chiral environmental contaminants to date (Bethan et al. 1997; Ridal et al. 1997; Ulrich and Hites 1998; Vetter et al. 1999; Garrison et al. 2000). Although most of these compounds were banned in the 1980s if not before, their persistence in the environment makes them interesting to study even today (Kurt-Karakus et al. 2007). Enantiomer analysis of these chemicals is often difficult due to the complex mixtures of their technical products. Many of the 32,768 theoretically possible configurations of toxaphene are chiral; chromatographic separation of single compounds in the mixture is very challenging, and avoiding coelutions for stereoisomer separations is also difficult (Vetter 1993). Despite this hurdle, chiral gas chromatographic separation has been accomplished for a few congeners in this complex pesticide mixture (Vetter et al. 1997; Kallenborn and Hühnerfuss 2001).

The organophosphorus pesticides were developed in the 1950s and have been used against plant diseases, insects, and weeds (Sasaki 1998). Their mode of action as an insecticide typically is through acetylcholine esterase inhibition (Kurihara et al. 1997). This group of pesticides is particularly interesting from a stereochemical standpoint, because a chiral center may be present at a phosphorus atom, carbon atom, or even at a sulfur atom. Some OP compounds are converted into oxon degradation products by replacing the phosphorus bonded sulfur atom with oxygen (Lee et al. 1978; Nomeir and Dauterman 1979; Hirashima et al. 1989; Berkman et al. 1993). These oxon degradates are often the more toxic and insecticidally active form, and some are chiral [fonofos oxon, EPN oxon (*O*-*e*thyl *O*-*p*-*n*itrophenyl phenylphosphonothioate), malaoxon, salioxon, etc.]. The efficacy of the stereoisomers depends on their structure, with phosphorus chirality making a greater impact on the variation in activity than does carbon chirality (Williams 1992; Buser and Francotte 1997). The difference between stereoisomer activities ranges from a factor of 1.5–20 or more (Sasaki 1998).

The synthetic pyrethroid insecticides are variants of the natural products found in chrysanthemum flowers. These compounds are neurotoxic, causing knockdown and mortality effects (Vijverberg and Oortgiesen 1988). Since allethrin, the first pyrethroid, was developed in 1949, advances have been made to make these compounds more photostable, less toxic to mammals, and good alternatives to more toxic legacy pesticides (Williams 1992). Pyrethroids usually have several chiral centers, often at the cyclopropane ring, creating multiple stereoisomers that have varying degrees of toxicity (Chamberlain et al. 1998). The nomenclature for this class lacks uniformity and is sometimes confusing, because single or enriched stereoisomer formulations often have names similar to those of compounds having unspecified stereochemistry. For example, fenvalerate is a racemic mix of four stereoisomers, whereas *es*fenvalerate is ≥75% resolved (*S*,*S*)-isomers; resmethrin is 20–30% (1*RS*)-*cis*- and 80–70% (1*RS*)-*trans*-isomers, whereas *bio*resmethrin is ≥90% (1*R*)-*trans*-isomer and ≤3% *cis*-isomers; a similar formulation pattern exists for cypermethrin and α-, β-, θ-, or ζ-cypermethrin. Additionally, the cyano group of the type II pyrethroids is not stereochemically stable, and can invert/isomerize under high temperature or in polar protic solvents with light (Ruzo et al. 1977; Liu et al. 2005; Qin and Gan 2007). This inversion can be problematic for chiral analyses, because liquid or supercritical fluid chromatography often utilize protic solvents, like alcohols, as part of the mobile phase.

ChirBase contains entries for 50 of the 149 insecticides (34%, see Table 4). There are 1,066 records for these 50 pesticides (706 in LC, 344 in GC, and 16 in CE databases). *d*-Limonene has the most entries (175 total) with all but seven found in the GC database. Several OC, OP, and pyrethroid insecticides (such as chlordane, phenthoate, and allethrin) are among those in ChirBase. These insecticides have been used extensively, are well studied by achiral techniques, and have successful enantioselective separation techniques developed and ready to use. However, Chirbase does not have any GC entries for fipronil, although it has been separated using enantioselective chromatography (Konwick et al. 2005).

4.4 Miscellaneous Other Pesticides

In Table 5, we list 95 other miscellaneous pesticides and note the primary pesticidal uses of each. Their structures are shown in Fig. 6. This grouping includes acaricides, bactericides, bioirritants, chemosterilants, insect attractants, insect pheromones, insect repellents, insecticide synergists, mammal repellents, nematicides, other, rodenticides, and virucides. The majority of these compounds have one to six chiral features, but there are several avermectin pesticides that have up to 20 chiral features. The following compounds have a chiral phosphorus atom in their structure: amidothioate, fenamiphos, fosthiazate, imicyafos, isamidofos, phosphocarb, and trifenofos. The following compounds have a chiral sulfur atom in their structure: acetoprole, aramite, fensulfothion, propargite, and sulfoxide.

Table 5 Miscellaneous chiral pesticides

Pesticide name	Chiral features (constrained)	*Meso*?	Alternate names, isomers	Salts, esters	CAS® #s	Type[a]	CE	GC	LC
1,2-*Dib*romo-3-*c*hloro*p*ropane	1		DBCP		96-12-8	N			
14-Methyloctadecene	1				93091-95-3	IP			
3,4-Dichlorotetrahydrothiophene 1, 1-dioxide	2	Y			3001-57-8	N			
4-Methylnonan-5-ol w/4-methylnonan-5-one	2 + 1				154170-44-2, 35900-26-6	IP		1	
5-(1,3-Benzodioxol-5-yl)-3-hexylcyclohex-2-enone	1		Piperonyl cyclonene		119-89-1	IS			
6-Methylhept-2-en-4-ol	1				4798-62-3	IP		1	
Acetoprole	1				209861-58-5	A			
α-Chlorohydrin	1				96-24-2	R		26	56
Acrinathrin	3				101007-06-1, 103833-18-7	A	2		4
Amidothioate	1				54381-26-9	A			
α-Multistriatin	4 (2)				59014-03-8	IA		1	
Aramite	2				140-57-8	A			
Brevicomin	3				20290-99-7	IR		35	
Brodifacoum	2				56073-10-0	R			
Bromadiolone	2				28772-56-7	R			
*B*utoxy(poly*p*ropylene *g*lycol)	Varies 2+[b]		BPG 400		9003-13-8	IR			
Cadusafos	2	Y	Ebufos		95465-99-9	N			
Chalcogran with methyl decadienoate	2				38401-84-2, 70427-57-5, 53172-59-1, 4493-42-9	IP		10	
Chloralose	6 (2)		Alphachloralose, glucochloralose		15879-93-3, 16376-36-6	R			
Chlorophacinone	1		Rozol	-Sodium	3691-35-8	R			1

(continued)

Table 5 (continued)

Pesticide name	Chiral features (constrained)	*Meso*?	Alternate names, isomers	Salts, esters	CAS® #s	Type[a]	CE	GC	LC
Closantel	1				57808-65-8	A			
Coumachlor	1				81-82-3	R	2		50
Coumafuryl	1		Fumarin, tomarin		117-52-2	R			13
Coumatetralyl	1				5836-29-3	R			
Cyflumetofen	1				400882-07-7	A			
DCIP	2	Y	*Di*chloro di*isop*ropyl ether		108-60-1	N			
Dicofol	1		**o,p'**-[c]		115-32-2	A			1
Dicyclopentadiene	4 (4)				77-73-6	MR			5
Difenacoum	2				56073-07-5	R			
Difethialone	2				104653-34-1	R			
Dimethyl carbate	4	Y			39589-98-5	IR			
Dinobuton	1				973-21-7	A			
Dinocton	2				32534-96-6, 19000-58-9, 19000-52-3, 32535-08-3, 6465-51-6, 6465-60-7, 104078-12-8	A			
Dinopenton	1				5386-57-2	A			
Dinosulfon	1				5386-77-6	A			
Disparlure	2				29804-22-6, 54910-51-9, 54910-52-0	IA			
Dominicalure	1 each				80510-16-3, 80510-15-2	IA			
Doramectin	19 (2)				117704-25-3	A			
ENT 8184	5 (4)		Zengxiaoan		113-48-4	IS			

Eprinomectin	19/20 (2)	B_{1a}, B_{1b}	123997-26-2, 133305-88-1, 133305-89-2	A		
Ergocalciferol	6 (2)	Vitamin D_2, calciferol	50-14-6	R		
Ethohexadiol	2		94-96-2	IR		
Ethyl-4-methyloctanoate	1	Oryctalure	56196-53-3	IP	2	
Etoxazole	1		153233-91-1	A		
Farnesol with nerolidol	1		4602-84-0, 3790-71-4, 7212-44-4	BI	48	
Fenamiphos	1		22224-92-6	N		18
Fenpropathrin	1	Danitol	64257-84-7, 39515-41-8	A	2	26
Fensulfothion	1		115-90-2	N		3
Flocoumafen	2		90035-08-8	R		
Fosthiazate	2		98886-44-3	N		
Frontalin	2 (2)		28401-39-0	IA	11	
Grandlure	2	**-I**[c], -II, -III, -IV, luretape	11104-05-5, 26532-22-9, 30820-22-5, 26532-23-0, 26532-24-1, 26532-25-2	IA		
Hexythiazox	2		78587-05-0	A		1
Icaridin	2	Picaridin, KBR 3023, propidine	119515-38-7	IR		
Imicyafos	1		140163-89-9	N		
Ipsdienol with (*S*)-*cis*-verbenol	1 + 3 (2)		14434-41-4, 18881-04-4, 35628-00-3	IA	10	13
Ipsenol	1		35628-05-8	IA	6	3
Isamidofos	1		66602-87-7	N		
Ivermectin	19/20 (2)	B_{1a}, B_{1b}	70288-86-7, 70161-11-4, 70209-81-3	A		

(continued)

Table 5 (continued)

Pesticide name	Chiral features (constrained)	*Meso*?	Alternate names, isomers	Salts, esters	CAS® #s	Type[a]	CE	GC	LC
Japonilure	1		Nuranone		64726-91-6, 64726-93-8	IA			
Lineatin	4 (4)				65035-34-9	IA		5	1
MB-599	1		Verbutin		185676-84-0	IS			
Medlure	4 each				13929-18-5	IA			
Metepa	3				57-39-6	CS			
Methiotepa	3				76-96-0	CS			
Methyl apholate	6				3527-55-7	CS			
Milbemectin	10 (2) each		-A3, -A4		51596-10-2, 51596-11-3	A			
Milbemycin oxime	9 (2) each				Not listed	A			
Moxidectin	10 (2)				113507-06-5	A			
Nikkomycin	8 each		-X, -Z		59456-70-1	A			
Nonanol	1				3452-97-9	O			
Norbormide	5 (4)		Nobormide		991-42-4	R			
Oxytetracycline	6 (3)		Terramicin, terramycin	-Hydrochloride	79-57-2, 2058-46-0	B			
Phosphocarb	1				126069-54-3	N			
Polynactins	16 each		Tetra-, tri-, di-nactin		33956-61-5, 7561-71-9, 20261-85-2, 39285-04-6	A			
Propargite	3		BPPS, 1-*tert-b*utyl-4-(2-*prop*-2-ynoxy-*s*ulfanyloxycyclohexoxy) benzene; formaldehyde		2312-35-8	A			8
Propyl isome	3				83-59-0	IS			
Ribavirin	4				36791-04-5	V			
S421	2	Y	Octachlorodipropyl ether		127-90-2	IS			
Scilliroside	13 (5)		Red squill		507-60-8	R			

Selamectin	14 (2)				165108-07-6	A			
Sesamex	1				51-14-9	IS			
Sesamolin	4 (2)	Y			526-07-8	IS			
Siglure	3				2425-20-9	IA			
Sordidin	4 (2)		7-Epi-sordidin	-A, -B	162490-88-2, 162428-76-4,	IP		1	
Streptomycin	15			-Sesquisulfate	57-92-1, 3810-74-0	B			
Strychnine	6 (6)				57-24-9	R			
Sulcatol	1				4630-06-2	IA		19	
Sulfoxide	2				120-62-7	IS			
Thuringiensin	13				23526-02-5	A			
Trifenofos	1				38524-82-2	A			
Trimedlure	6		-A, -B1, -B2, -C		12002-53-8, 5748-22-1, 5748-20-9, 5748-21-0, 5748-23-2	IA			
Verbenone	2 (2)				18309-32-5, 1196-01-6, 80-57-9	IP		6	14
Vitamin D3	5 (2)				67-97-0	R			
Warfarin	1		Coumafène, zoocoumarin	-Potassium, -sodium	81-81-2, 5543-58-8, 5543-57-7, 2610-86-8, 129-06-6	R	67		557

[a]Primary pesticidal action: A = acaricide, B = bactericide, BI = bioirritant, CS = chemosterilants, IA = insect attractant, IP = insect pheromone, IR = insect repellent, IS = insecticide synergist, MR = mammal repellent, N = nematicide, O = other, R = rodenticide, V = virucide

[b]Butoxypolypropylene glycol is a polymer, therefore, the number of chiral features will depend on the length of the polymer. The base propylene glycol unit has two chiral features

[c]Isomers listed in bold refer to the chiral form

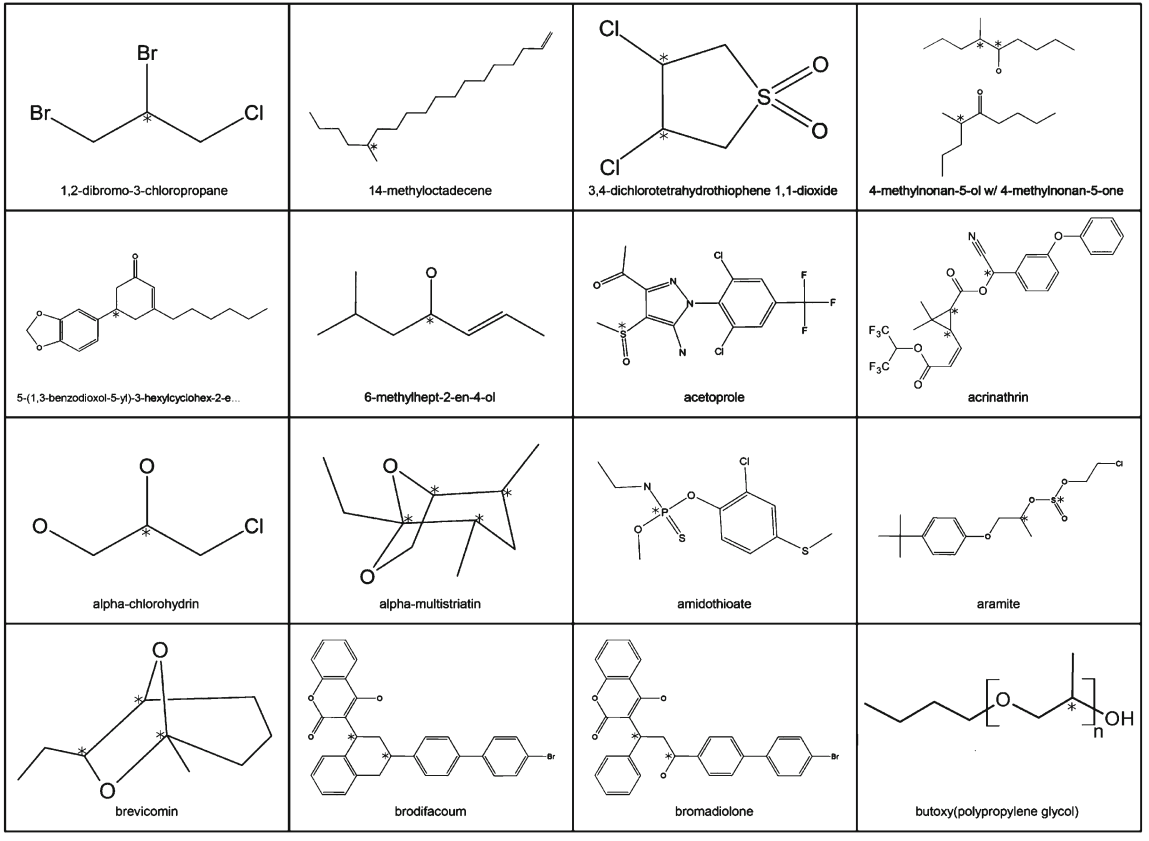


Fig. 6 Miscellaneous chiral pesticide structures. See Fig. 2 for symbol description

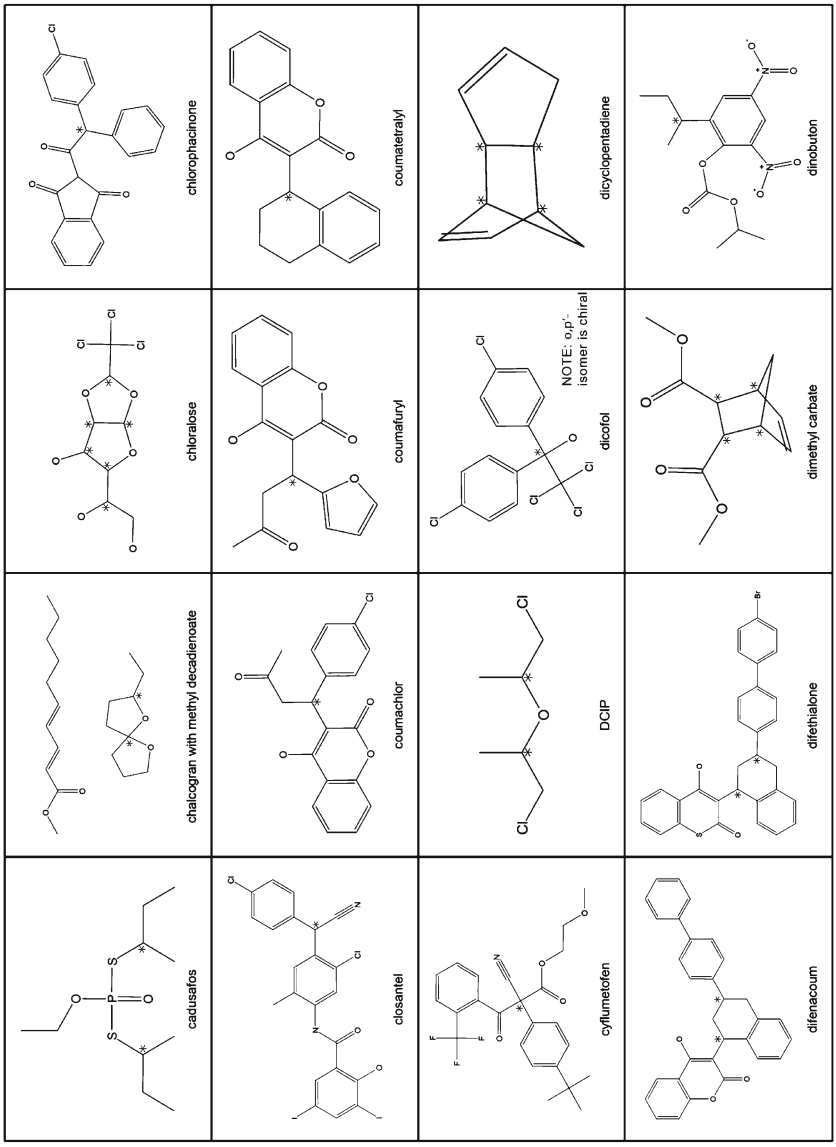


Fig. 6 (continued)

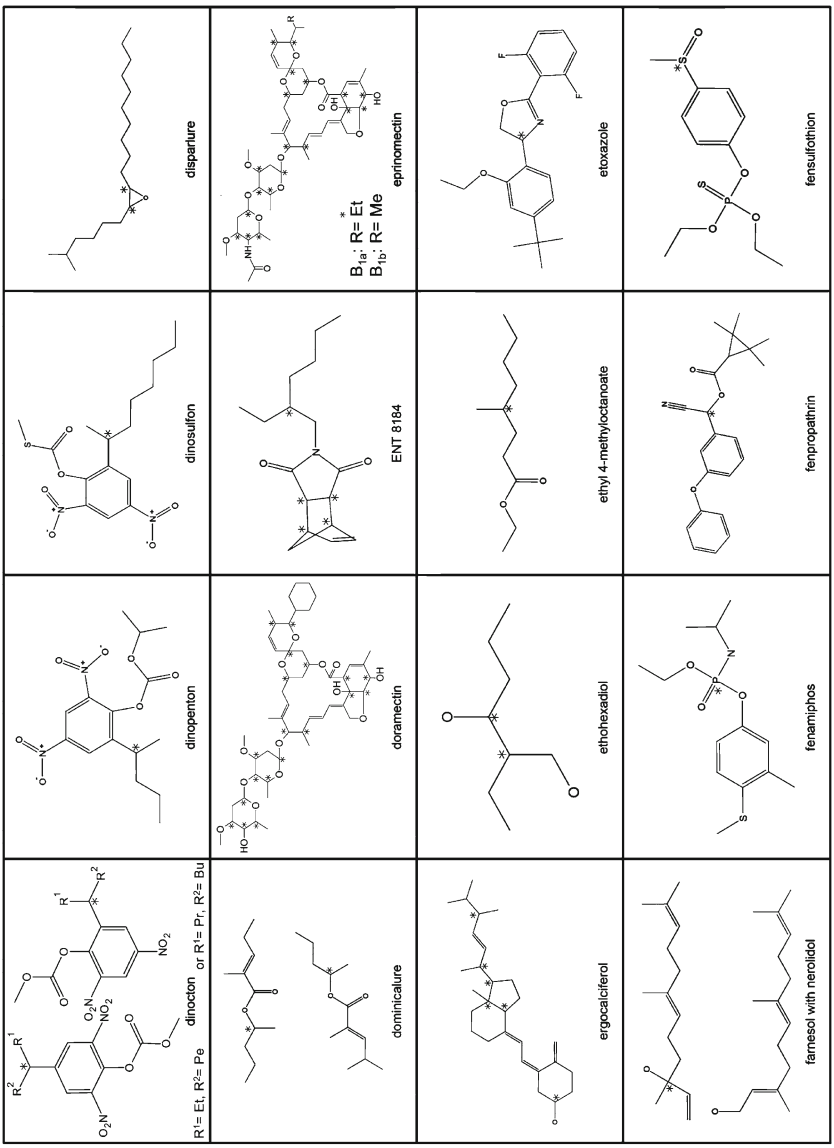


Fig. 6 (continued)

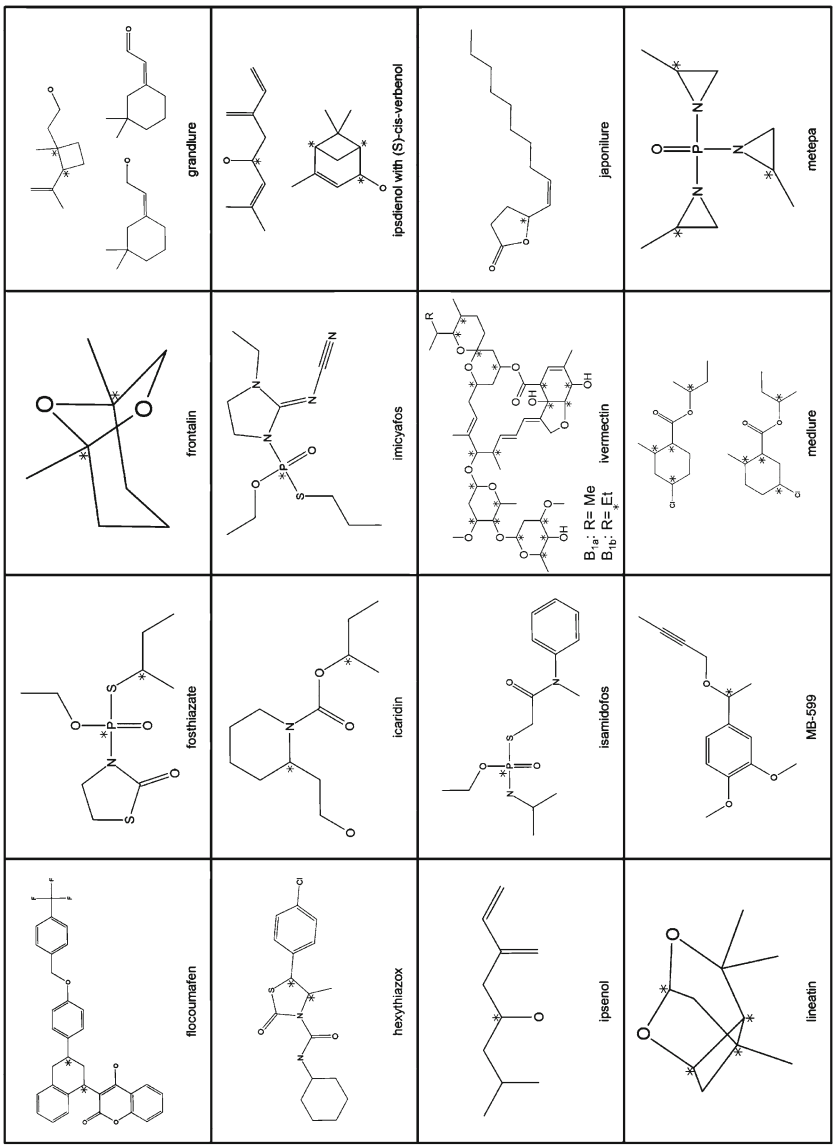


Fig. 6 (continued)

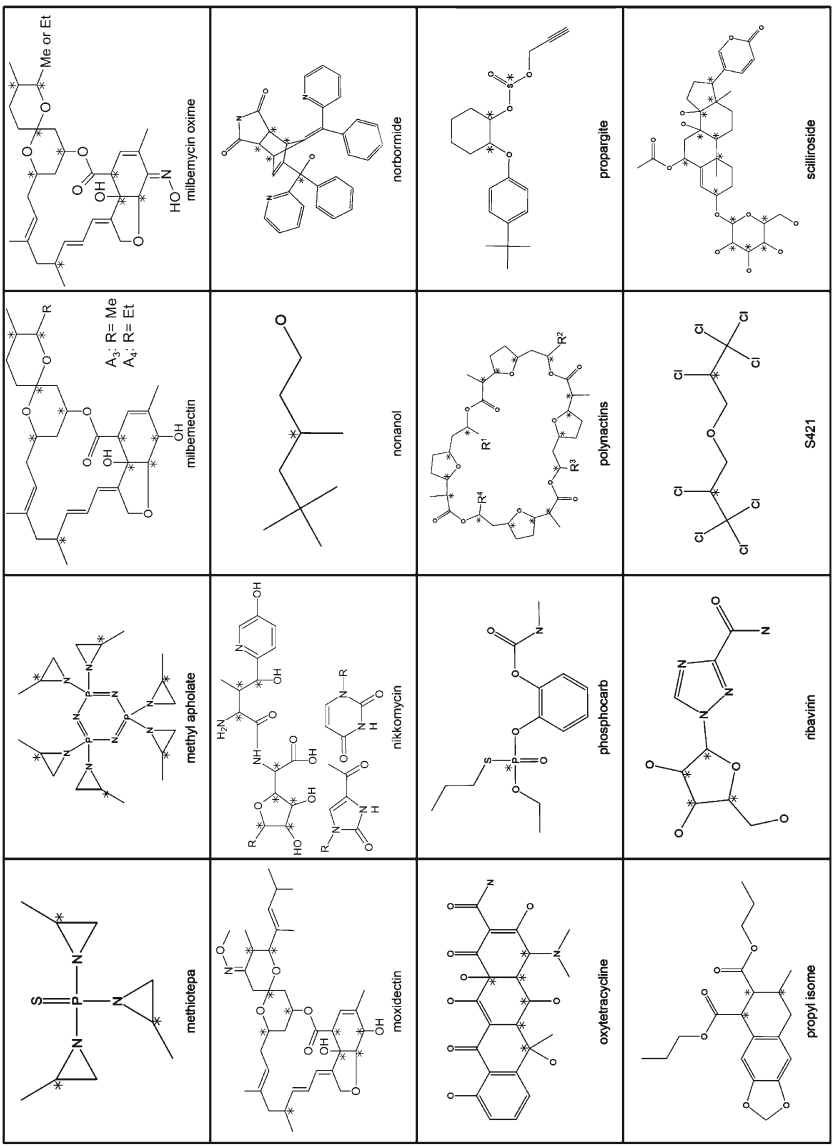
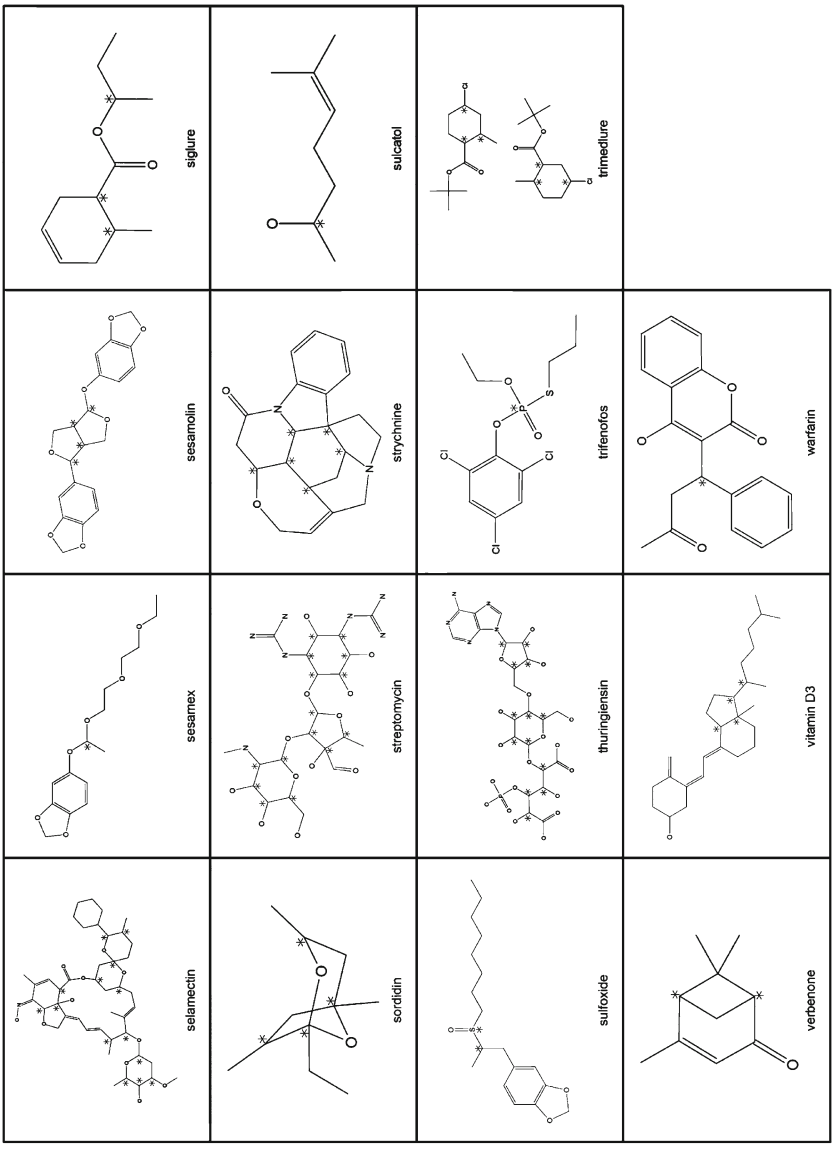


Fig. 6 (continued)


siglure
sesamolin
sesamex
selamectin
sulcatol
strychnine
streptomycin
sordidin
trimedlure
trifenofos
thuringiensin
sulfoxide
warfarin
vitamin D3
verbenone

In Fig. 6, all possible chiral centers have been marked, but for compounds with fused rings, there will be fewer possible stereoisomers. Compounds that have such constraints or *meso* forms are noted in Table 5. Because some of the large molecules are produced biologically, these chemicals probably are produced as single stereoisomers rather than as mixtures. No additional single or enriched formulations are noted in Table 5.

Although the insect attractants, pheromones, and repellents are fairly simple molecules, stereochemistry plays a particularly important role, because these compounds commonly interact with specific biological molecules (Borden et al. 1976; Vité et al. 1976; Payne et al. 1982; Mori 1997). For example, *Dendroctonus brevicomis* (western pine beetle) was more attracted to the (+) enantiomer of exo-brevicomin and the (−) enantiomer of frontalin than to their antipodes. There were also sex differences, with females exhibiting greater attraction to all exo-brevicomin treatments, especially the (−) enantiomer [the male/female ratio attracted was 0.92 for the racemic mixture, 0.85 for the (+) enantiomer, and 0.58 for the (−)] (Wood et al. 1976). Population and species differences have also been noted; *Ips pini* East (pine engraver) were attracted more to a mixture of ipsdienol enantiomers, whereas *Ips pini* West were attracted more to (−)-ipsdienol. *Ips paraconfusus* (California fivespined) was attracted more to (+)-ipsdienol, when in combination with other pheromone components (Mustaparta et al. 1980).

ChirBase contains entries for 27 of the 95 miscellaneous pesticides (28%, see Table 5). There are 1,029 records for these 27 pesticides (774 in LC, 182 in GC, and 73 in CE databases). Warfarin has the most entries (624 total), with most of these in the LC database. Warfarin, an anticoagulant rodenticide, is used pharmaceutically as the blood thinner coumadin. Because warfarin has medicinal uses, much is known about the efficacy (Eble et al. 1966), separation (De Vries and Völker 1989), and metabolism (Park 1988) of its enantiomers. Again, ChirBase does not contain all published stereoisomer separations, so readers are encouraged to perform a thorough literature search for chromatographic methods prior to undertaking research on any specific chiral pesticide.

5 Summary

Of the 1,693 pesticides considered in this review, 1,594 are organic chemicals, 47 are inorganic chemicals, 53 are of biological origin (largely nonchemical; insect, fungus, bacteria, virus, etc.), and 2 have an undetermined structure. Considering that the EPA's Office of Pesticide Programs found 1,252 pesticide active ingredients (EPA Pesticides Customer Service 2011), we consider this dataset to be comprehensive; however, no direct comparison of the compound lists was undertaken. Of all pesticides reviewed, 482 (28%) are chiral; 30% are chiral when considering only the organic chemical pesticides. A graph of this distribution is shown in Fig. 7a. Each pesticide is classified with up to three pesticidal utilities (e.g., fungicide, plant growth regulator, rodenticide, etc.), taken first from the Pesticide Manual as a

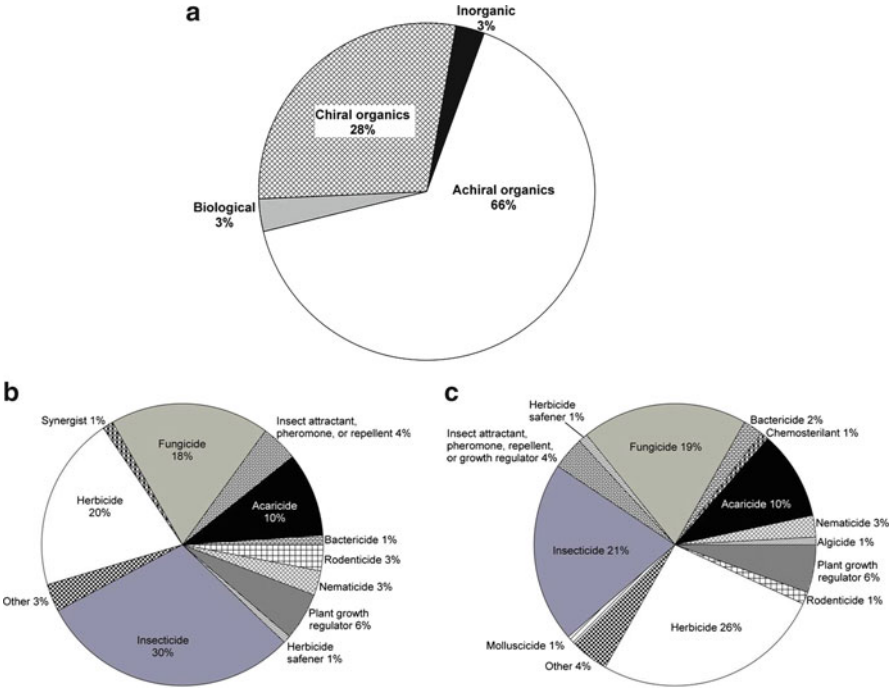


Fig. 7 Pesticide type (chiral and achiral organic, inorganic, biological) and utility statistics. (**a**) General pesticide type for 1,693 dataset entries. (**b**) 482 chiral pesticides by specific utility. (**c**) 1,211 achiral pesticides by specific utility. All definitions are adapted from IUPAC's Compendium of Chemical Terminology—the Gold Book (IUPAC 2006)

primary source, and the Compendium of Common Pesticide Names website as a secondary source. Of the chiral pesticides, 195 (34%) are insecticides (including attractants, pheromones, and repellents), 150 (27%) are herbicides (including plant growth regulators and herbicide safeners), 104 (18%) are fungicides, and 55 (10%) are acaricides. The distribution of chiral pesticides by utility is shown in Fig. 7b, including categories of pesticides that make up 3% or less of the usage categories. Figure 7c shows a similar distribution of nonchiral pesticide usage categories. Of the chiral pesticides, 270 (56%) have one chiral feature, 105 (22%) have two chiral features, 30 (6.2%) have three chiral features, and 29 (6.0%) have ten or more chiral features.

Chiral chemicals pose many difficulties in stereospecific synthesis, characterization, and analysis. When these compounds are purposely put into the environment, even more interesting complications arise in tracking, monitoring, and predicting their fate and risks. More than 475 pesticides are chiral, as are other chiral contaminants such as pharmaceuticals, polychlorinated biphenyls, brominated flame retardants, synthetic musks, and their degradates (Kallenborn and Hühnerfuss 2001; Heeb et al. 2007; Hühnerfuss and Shah 2009). The stereoisomers of pesticides can

have widely different efficacy, toxicity to nontarget organisms, and metabolic rates in biota. For these reasons, it is important to first be aware of likely fate and effect differences, to incorporate molecular asymmetry insights into research projects, and to study the individual stereoisomers of the applied pesticide material.

With the advent of enantioselective chromatography techniques, the chirality of pesticides has been increasingly studied. While the ChirBase (Advanced Chemistry Development 1997–2010) database does not include all published chiral analytical separations, it does contain more than 3,500 records for 146 of the 482 chiral pesticides (30%). The majority of the records are found in the liquid chromatography database (2,677 or 76%), followed by the gas chromatography database (652 or 18%), and the capillary electrophoresis database (203 or 6%). The finding that only 30% of the chiral pesticides covered in this review have entries in ChirBase highlights the need for expanded efforts to develop additional enantioselective chromatographic methods. Other techniques (e.g., nuclear magnetic resonance and other spectroscopy) are available for investigation of chiral compounds, but often are not utilized because of cost, complexity, or simply not recognizing that a pesticide is chiral.

In this review, we have listed and have briefly described the general nature of chiral fungicides, herbicides, insecticides, and other miscellaneous classes. A dataset generated for this review contains 1,693 pesticides, the number of enantioselective separation records in ChirBase, pesticide usage class, SMILES structure string and counts of stereogenic centers. This dataset is publically available for download at the following website: http://www.epa.gov/heasd/products/products.html. With the information herein coupled to the publically accessible dataset, we can begin to develop the tools to handle molecular asymmetry as it applies to agrochemicals. Additional structure-based resources would allow further analysis of key parameters (e.g., exposure, toxicity, environmental fate, degradation, and risks) for individual stereoisomers of chiral compounds.

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Strategies for Chromium Bioremediation of Tannery Effluent

Satyendra Kumar Garg, Manikant Tripathi, and Thiruneelakantan Srinath

Contents

1 Introduction

Pollution of the environment by toxic metals results largely from industrial activities, although sources such as agriculture and sewage disposal also contribute to some extent (Nriagu and Pacyna 1989). Toxic metallic species, once mobilized into the environment, tend to persist, circulate, and eventually accumulate at different trophic levels in members of the food chain. Ultimately, metal pollutants pose a serious

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threat to the environment, and affect plants, animals, and humans (Olson and Foster 1956; Reidske 1956; Sauter et al. 1976; Levis and Bianchi 1982; Mance 1987; Xing and Okrent 1993). Metal pollutants eventually affect ecosystem function, and impose an economic and public health burden. The problems associated with wastewater disposal in developing countries can generally be attributed to lack of adequate treatment/management policies, coupled with ineffective legislation on the part of entrusted governmental agencies (The Environmental Protection Act 1991; Oboh and Aluyor 2008). Environmental awareness has grown among consumers and industrialists in recent decades, and more recently has culminated in legal constraints being imposed on emissions; such constraints have increasingly become more strict necessitating cost-effective emission control (Gadd and White 1993).

The extensive use of hexavalent chromium in diverse products and processes of various industrial applications has caused substantial environmental contamination (Nriagu 1988a, b; Viti et al. 2003; Sultan and Hasnain 2007). Tanning industries convert animal skins and hides into leather, and in so doing, employ chromium compounds extensively, particularly chromium sulfate. The discharge of chromium-laden effluent has contaminated soils, sediments, and surface and ground waters (Szulczewski et al. 1997). Other major sources of chromium pollution derive from metal finishing, petroleum refining, iron and steel production, inorganic chemicals production, textile manufacturing, and pulp-producing industries (French et al. 1997; Mukherjee 1998; Chirwa and Wang 2000). In addition, chromium compounds are used in nuclear power plants, added to industrial cooling waters to inhibit corrosion, and also used as constituents of some wood preservatives (Patterson 1985). Chromium wastes are also associated with the metal plating and painting industries. Chromium waste is released into the environment via leakage, poor storage, or improper treatment and disposal practices (Calder 1988; Palmer and Wittbrodt 1991). Industries, such as those described above, release Cr^{6+} at levels ranging from 40 to 25,000 mg L^{-1} of wastewater (Sag and Kustal 1996a, b; Ganguli and Tripathi 1999). Because of its toxicity, carcinogenicity (One 1988; Yassi and Nieboer 1988), teratogenicity (Abbasi and Soni 1984), and mutagenicity (Morris et al. 1988; Anttila 1990; Lee et al. 2008), the United States Environmental Protection Agency (US EPA) has designated chromium as a priority pollutant (Roe and Carter 1969; Enterine 1974).

The tanning industry commonly employs "chrome liquor" in the tanning process, and discharges effluent into the environment that contains chrome salts in excess of the maximum permissible limits. In India, the standard limit for Cr^{6+} discharge in inland surface waters is 0.1 mg L^{-1} (IS: 2296; IS: 2490) (Bhide et al. 1996). The similar value established for the USA by the US EPA is 0.05 mg L^{-1} (U.S. EPA 1979). Despite the fact that, in trace quantities, chromium is an essential nutrient for humans, setting such permissible limits are essential, because at elevated levels Cr is toxic (Mertz 1974; NAS 1974; Lee et al. 2008).

In India, there are approximately 3,000 tanneries in existence, and collectively they employ more than 2.5 million people. Nearly 80% of these tanneries are engaged in tanning processes that utilize chrome (Rajamani et al. 1995). Approximately 80 million hides and 130 million skin pieces are processed annually. Export of leather goods has recently reached a new high of $2.8 billion (Rs. 14,000 crores) (Amudeswari et al. 2009). Approximately 50 L of effluent is generated per kg of skin/hide processed.

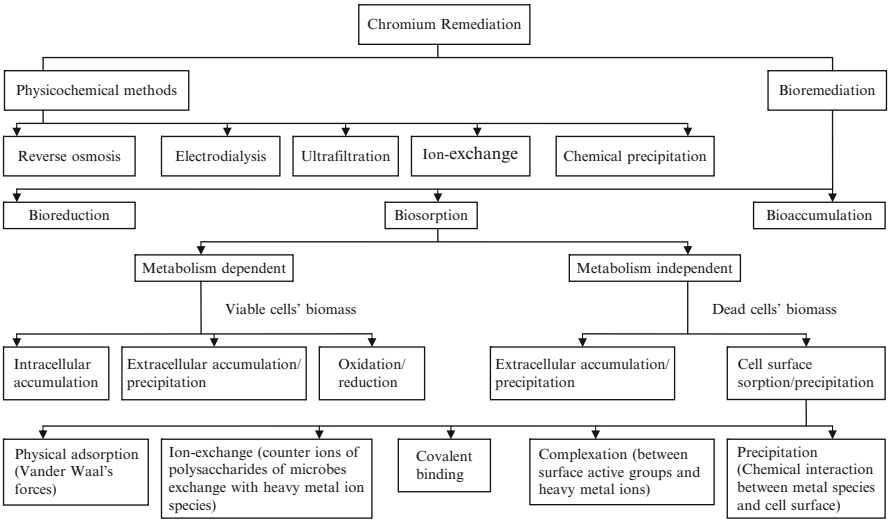

Fig. 1 Different treatment techniques contributing to chromium remediation from industrial effluents

In India, of the total chromium effluent discharged >50% originates from the leather, iron and steel industries. Rather than discharging such pollutants, a better approach is to prevent the pollution, if possible, or at least transform the pollutants to innocuous substances, before they are released. Currently, physicochemical and biological wastewater treatment plants are operated for the purpose of rendering the effluent from the tanning industries safe. A schematic diagram showing the different approaches for treating tanning effluent is presented in Fig. 1.

The plants that treat tannery effluent utilize physicochemical methods to detoxify Cr^{6+}. Such methods include chemical reduction, precipitation, filtration, electrochemical treatment, membrane technologies, evaporation recovery, solvent extraction, ion exchange, reverse osmosis, and adsorption on activated carbon (Hafez et al. 2002; Esmaeili et al. 2005; Ahluwalia and Goyal 2007). Unfortunately, in developing countries, none of these methods have achieved economic viability. Attempts have been made to use conventional biological treatment plants (aerobic as well as anaerobic) to achieve safe disposal of tannery wastewater. Although such conventional plants achieved the goal of removing heavy metal(s) from effluent to some extent, they created another costly disposal problem, i.e., they generated heavily laden metal sludge. Hence, conventional methods for removing Cr^{6+} from wastewaters are often cost prohibitive. These constraints have prompted an ongoing search for alternative treatment technologies that rely on metal sequestration, a more cost-effective and environmentally acceptable bioremediation approach.

Bioremediation is a process in which microorganisms are used to reduce or eliminate an undesirable chemical contaminant. Biotechnological approaches to abating toxic metal pollutants consist of selectively using natural processes to treat particular wastes. The processes by which microorganisms interact with toxic metals to enable their removal and recovery are called "biosorption" and "bioaccumulation"

(Pattanapipitpaisal et al. 2002; Rehman et al. 2007; Sultan and Hasnain 2007). The major advantages of bioremediation over conventional physicochemical and biological treatment methods include low cost, good efficiency, minimization of chemicals, reduced amounts of secondary sludge, regeneration of biosorbent, and the possibility of recovering the pollutant metal(s). Research on the use of microbes for bioremediation of toxic metal(s) has largely derived from laboratory-based experiments (Lange et al. 1998; Tucker et al. 1998). Field-related studies that utilize microorganisms for toxic metal remediation have been more sparse, but are now becoming more prevalent. However, when undertaking field-scale bioremediation studies, it is essential to conduct pilot scale research for the purpose of evolving an appropriate management strategy, before starting actual field bioremediation. Fortunately, bioremediation is an eco-friendly treatment strategy, is a cost-effective process, and is gradually making inroads as a key way to effect environmental clean-up.

The purpose of this review is to compile the scattered information on different strategies for treating tannery waste, viz., biosorption, bioaccumulation, bioreduction, and immobilization of biomass for chromium bioremediation.

2 History of Tannage

Leather making has been known to man since prehistoric times, when hides and skins were essential materials for construction of garments, worn either for protection or for other utilitarian purposes. However, the understanding of the scientific principles behind leather making came much later. As tanners gained insights into how the process worked, newer processes and methodologies for making leather softer were created (Amudeswari et al. 2009).

Tanning is an ancient art. Tanning is the means by which putrescible animal hides and skins are preserved from decay, and are converted into an imputrescible material, known as leather. In archaeological excavations, ancient leather articles have been unearthed in very sound condition; it has been estimated that some unearthed leathers are approximately 12,000 years old (Sarkar 1991). In an attempt to render the skin soft, prehistoric people at some point laid the foundation for "the art of tanning," in which skin was greased and dried after the application of fatty/albuminous matters such as tallow, oil, egg yolk, milk, and curd. It is of interest that the Hebrews of Biblical times used oak bark for tanning, and the Romans, Greeks, and Egyptians used lime water to loosen the hair on hides and skins; these two processes are still in use today (Findlay 1934; Sarkar 1991).

Despite the fact that tannery is an ancient art, it was not until the end of the eighteenth century that this process attracted the attention of scientific man. It is now recognized that tanning is a complex phenomenon in which chemical, physical, and bacteriological principles are involved. Despite the advances made by modern science, leather manufacture is still an art, involving multiple manipulations and adjustments in its associated processes to produce leather of good quality.

3 Chrome Tanning Processes

There are two major types of tanning systems, viz., vegetable tanning and chrome tanning. The former causes a high pollution load and low treatability, so it is not considered to be an environmental friendly option. Furthermore, vegetable-tanned leathers have different physical properties and produce a biodegradable form of leather (Moore and Ramamoorthy 2001; Belay 2010). Hence, chrome tanning has been the more common method employed for processing hides (Sreeram and Ramasami 2003). Chromium salts, particularly chromium sulfate, is the most commonly employed tanning agent today. Tanning agents help stabilize the skin matrix against biodegradation and render it more permanent. Hides tanned with chromium salts have good mechanical strength, an extraordinary dying suitability and better hydrothermic resistance, when compared to vegetable tanned hides (Belay 2010).

Elemental chromium (Cr) does not occur in nature, but is present in ores, primarily in the form of chromite ($FeOCr_2O_3$). Chromium exists in oxidation states ranging from +6 to −2 (Avudainayagam et al. 2003), although only the +6 and +3 oxidation states are commonly encountered in the environment (Cervantes et al. 2001; Megharaj et al. 2003; Nath et al. 2009). Cr^{6+} exists in solution as the monomeric ions $H_2CrO_4^0$, $HCrO_4^-$ (bichromate), and CrO_4^{2-} (chromate), or as the dimeric ion $Cr_2O_7^{2-}$ (dichromate) (Palmer and Wittbrodt 1991; Richard and Bourg 1991).

Chromium was discovered by Nicolas-Louis Vauquelin in 1797, in Siberian red lead ore (crocoite $PbCrO_4$). Fredrick Knapp is credited with the development of the modern practice of chrome tannage (Cotta 1921; McLaughlin and Theis 1945; Wilson 1948). The two-bath tanning process was patented by August Schultz (McLaughlin and Theis 1945) in 1884, and he was the first to apply chromium compounds for the commercial production of leather. The one-bath chromium tannage process, which is widely used today, was patented in 1893 by Martin Dennis. Owing to its light weight, durability, and resistance to heat and water, chrome-tanned leather, which is also easy to dye and finish, is popular among customers. Use of chromium in tannery emphasizes its leather conservation properties, because it confers to the finished leather great water proofing, high stability, easy handling, flexibility, and shorter overall processing time (Maria et al. 1999). The chromates used in the two-bath and single-bath processes of the leather industry (Thorstensen 1958) do not have tannage properties as such. They are, however, reduced to Cr^{3+}, after penetration into skins (two-bath process), or before contacting the skin (one-bath process). Sodium thiosulfate is used to reduce chromate during the two-bath process, whereas corn sugar/sulfur dioxide is used in the one-bath process. The chromium, after reduction, becomes a sulfate compound [$Cr_2(SO_4)_3$], which does not have tanning properties (i.e., its three radicals are acidic). It is, however, possible to replace the acidic radicals with basic hydroxyl (OH^-) groups, which increases the basicity of the compound as under:

$$0\%\ [Cr_2(SO_4)_3] < 33.3\%\ [2Cr(OH)\ (SO_4)] < 66.7\%\ [Cr_2(OH_4)\ (SO_4)] < 100\%\ [2Cr(OH_3)].$$

The hide or skin fiber has the power of absorbing and permanently fixing the basic salts only, whereby the fiber is tanned. The increase in the number of OH^- groups held by basic chromium salts is, therefore, a decisive factor in the tanning action. There is an optimum range for the basicity of the liquor used for effective tanning. Generally, a too low basicity produces exceptionally smooth grains of leather, but empty, flat, thin, and somewhat under-tanned leather. In contrast, a too high basicity increases fixation and overloads the grain, thereby making it coarse, resulting sometime in a cracked-grain surface. The range of basicity used in the initial stage of tanning practice is generally 25–30% Schorlemmer (i.e., salts containing on an average less than 1 hydroxyl per atom of chromium; Sarkar 1991). After the completion of the tanning process, the resultant liquor has been confirmed to contain monovalent (Cr^{1+}) to pentavalent (Cr^{5+}) species of chromium (Venba et al. 1999).

4 Chromium-Laden Effluent Discharge

In India, more than 90% of hides and skins that are produced are processed annually in ~3,000 tanneries, and all of these are treated with one form of chromium or another. The annual use of chrome salts in India has been estimated to be 4,000 t, of which, 60–70% is used for tanning. About 30–40% of chromium used for tanning remains in the spent tanning liquor, which is normally sent to a wastewater treatment plant (Esmaeili et al. 2005). The untreated effluent emanating from the chrome tanning sectional waste contains 1,500–3,000 mg chromium L^{-1} wastewater (Rao et al. 1999). However, the present day high-exhaust chrome tanning method leads to wastewater levels in the effluent of 500–1,000 mg chromium L^{-1} (Aravindhan et al. 2004). In India, the discharge limit for chromium ranges from 1 to 5 mg L^{-1} for direct discharge to water bodies, and 1–20 mg L^{-1} for discharge to sewage systems. Therefore, the tanning industry's treatment plants require influents to be treated such that the Cr^{6+} levels are reduced by a factor of 200 times (to meet the discharge limit of 5 mg Cr^{6+} L^{-1}), before it is discharged to water bodies, and this is not easy to achieve (Tadesse et al. 2006). As stated above, nearly 80 million hides and 130 million skin pieces are processed annually by the tanning industry in India. The quantity of effluent discharged from this tanning activity equals 30–40 Lkg^{-1} skin/hide processed, and for finishing units, this quantity is approximately 50 Lkg^{-1} raw hide/skin processed (Agarwal 1996). The annual total discharge of tannery effluent in India is estimated to be 9.42×10^6 L (Srivastava and Pathak 1997).

To meet the challenge of chromium pollution resulting from tannery waste, a concerted effort has been undertaken that involves both better surveillance of chromium use, and improvements in in-plant and end-of-pipe treatment technologies. Presently, 60% of tannery effluent generated in India is treated by 250 individual and 60 common-effluent treatment plants (Buljan and Sahasranaman 1999). However, complete removal of chromium has not been achieved, and has evolved a new problem relating to safe disposal of metal-laden sludge. Regions where treated

effluent and sludge from treatment plants were disposed of onto arable land have resulted in a significant buildup of chromium content in the soil. Chromium waste, when disposed of via land application is also known to leach to ground water (Kumar et al. 1999; Sakthivel et al. 1999). The fact that more than 100 treatment units are not operating at optimum levels has further aggravated the situation, and has led to increased fear of pollution in the vicinity of treatment plants (Chemical Weekly 2001). The sludge generated in these treatment plants is not safe for land disposal due to the presence of high levels of metals and the associated toxicity of their leachates (Srinath and Ramteke 1999), and concern for metal accumulation in crops (Kwon et al. 1999; Sarvanan et al. 1999; Barman et al. 2000).

To reduce the rate of release of toxic chromium, the prospects of using spent liquor for retanning of leather have been studied. Its success in tanning of fresh skins depends on the residual chromium concentration present in the liquor (Rao et al. 1999). The use of spent liquor could potentially be applied to all tanneries, and especially to those in which leather quality depends on good tanning performances (Celma et al. 1999).

5 Toxicity of Chromium

An average person weighing 70 kg retains approximately 14 mg of chromium in his/her body (Emsely 1999). Only two oxidation states of chromium, viz., hexavalent and trivalent forms are stable in the environment. The biological interactivity of these two stable chromium forms varies considerably (Thacker et al. 2006; Gupta and Rastogi 2008; Kilic and Donmez 2008; Mishra and Doble 2008). Hexavalent chromium (Cr^{6+}) is highly mobile and water soluble, whereas Cr^{3+} is relatively inert, chemically more stable, and less bioavailable due to its negligible permeability through biomembranes (Myers et al. 2000; Megharaj et al. 2003; Pal et al. 2005). Cr^{6+} is nearly 100 times more toxic (Yao et al. 2008) and 1,000 times more mutagenic than Cr^{3+} (Barrera et al. 2008). Cr toxicity in humans normally occurs from exposures to environmental pollution via soil or water contamination, or from occupational exposure. Intoxication by Cr^{6+} causes serious morbidity and mortality. Even a slight elevation in the level of Cr^{6+} elicits environmental and health problems that derive from its high toxicity (Sharma et al. 1995), mutagenicity (Nishioka 1975), and carcinogenicity (Venitt and Levy 1974). Soluble Cr^{6+} poses a significant carcinogenic risk if ingested, because of the acidic pH of the stomach, which dissolves particulate chromate (Holmes et al. 2008; Ray and Ray 2009). Toxic and mutagenic effects of chromium on microorganisms have also been reported to occur at concentrations between 10 and 12 mg L^{-1}. These mutagenic effects are attributed to an alteration of genetic material, and altered metabolic and physiological reactions (Losi et al. 1994a).

Nonoccupational exposure to chromium occurs via ingestion of chromium-containing food and water, whereas occupational exposure results primarily through inhalation. Cr^{3+} is poorly absorbed, regardless of the route of exposure, whereas

Cr^{6+} is more readily absorbed (Ray and Ray 2009). Humans and animals accumulate chromium in various tissues that include lung, liver, kidney, spleen, adrenals, plasma, bone marrow, and red blood cells. The respiratory and dermal toxicity of chromium is well documented (Holmes et al. 2008). The painters Rubens, Renoir, and Dufy suffered from rheumatoid arthritis and Klee from scleroderma, and were the first persons who have suffered a documented "occupational exposure" to heavy metals (Pedersen and Permin 1988). These artists used heavy metals, including chromium, because these metals imparted bright and clear colors. The toxicity of Cr^{6+} is higher because of its high membrane permeability (Lovely and Coates 1997), which produces functional alteration of the lung, respiratory tract, liver, pancreas, and kidney. Gibb et al. (2000a, b) reported several ailments that were associated with Cr^{6+} exposure, which included nasal irritation and ulceration, skin irritation, eardrum perforation, and lung carcinoma. Hexavalent chromium accumulates in the placenta, and thereby impairs fetal development in mammals (Saxena et al. 1990).

Maria et al. (1999) found that workers exposed to chromium in the tanning process displayed several ailments pertaining to a diminution of general health. The observed effects included hypoglycemia, respiratory cancer, and nephritic ailments. Epidemiological studies on industrial workers exposed to Cr^{6+} disclosed a higher incidence of respiratory cancer than that occurred in the normal population (Norseth 1986; Langard 1990). Katz and Salem (1994) reported nasal mucous membrane perforation in exposed workers at tannery-, galvanoplastic-, and chromate-production units. Additionally, renal and hepatic toxicity were reported in workers exposed to Cr^{6+} (Love 1983; Verschoor et al. 1988), as were nephrotoxicity and hepatotoxicity (Appenroth and Kersten 1990; Standeven and Wetterhahn 1991; Ueno 1992) in experimental animals that resulted in DNA damage (e.g., single strand breaks and DNA–protein cross-links in cultured and in vivo cells; de Flora et al. 1990). Overexposure to Cr^{6+} reportedly produced allergic dermatitis, and ulceration of skin, mucous membranes, and the nasal septum, in addition to renal tubular necrosis and increased risk of respiratory tract cancer (Lu and Yang 1995; Flavio et al. 2004). Human dietary doses to Cr^{6+} greater than 10 mg kg^{-1} mainly affected the gastrointestinal tract, kidneys, and potentially the hematopoietic system. Although heavy metals are predominantly present in many industrial effluents, they often co-exist with other toxic organic and inorganic compounds. When this occurs, the mixtures can exert toxicity in a complex manner. For example, binary mixtures of free cyanide plus Cr^{6+} resulted in higher fish lethality than was predicted by either response- or concentration-addition models (Leduc et al. 1982).

Under normal physiological conditions, Cr^{6+} is believed to be reduced inside cells to form the more stable Cr^{3+}, and this reaction produces certain short-lived intermediates, such as Cr^{5+} and/or Cr^{4+} free-radicals (Camargo et al. 2003; Costa 2003; Xu et al. 2004, 2005; Pal et al. 2005; Cheung et al. 2006). Such reduction is achieved via cellular reductants such as glutathione, cysteine, ascorbic acid, riboflavin, and NADH-dependent flavoenzymes (e.g., microsomal cytochrome P_{450} reductase) (de Flora and Wetterhahn 1989; de Flora et al. 1990; Sugiyama 1992; Yuann et al. 1999). Therefore, the formation of paramagnetic species such as Cr^{5+}

may play an important role in the induction of toxicity by Cr^{6+}. In fact, Cr^{6+}/Cr^{5+}, Cr^{5+}/Cr^{4+}, and Cr^{3+}/Cr^{2+} oxidation/reduction couples have been shown to serve as cyclical electron donors in Fenton-like reactions, which generate active oxygen species (e.g., hydroxyl radicals), that are known to produce several toxic effects (Shi and Dalal 1990; Sugden et al. 1992; Luo et al. 1996; Shi et al. 1999). Ueno et al. (2001) reported that hydroxyl radicals, formed during Cr^{6+} reduction, may play an important role in the DNA strand breaks caused by this metal, and implied that the levels of Cr^{6+} inside the cells may not always be related to the induction of DNA strand breaks.

6 Chromium as a Micronutrient

In contrast to the toxicity exerted by Cr^{6+} (Lock and Janssen 2002; Feng et al. 2004; Park et al. 2004), more than 50 years of research has led to the recognition that Cr^{3+} is an essential oligoelement (Bailar 1997; Mertz 1998) in the glucose metabolism and lipid synthesis pathways (Anderson 1998). Trivalent chromium is considered to be a trace element required for the proper functioning of living organisms (Bahijri and Mufti 2002; Wang et al. 2009). Nutritionally, at lower concentrations, Cr^{3+} is an essential component of balanced human and animal diets for preventing adverse effects (e.g., impaired glucose tolerance, increased fasting insulin, increased cholesterol and triglycerides and hypoglycemic symptoms) in the metabolism of glucose and lipids (Zayed and Terry 2003). Cr^{3+} is also known to be a part of several enzymatic systems, and is related to a low molecular weight compound termed the glucose tolerance factor (GTF). The GTF interacts with insulin to promote the normal utilization of glucose (Ernest 1991). Shortly after Cr^{3+} was identified as an essential element in 1959, its interaction with insulin in vitro and in vivo was established, and its site of action was identified to be the insulin-sensitive cell membrane (Mertz 1998). It has also been demonstrated that beer yeast, which is rich in GTF, improved glucose utilization and reduced cholesterol and triglyceride levels in the serum of several aged subjects. GTF also reduced the need of insulin in certain diabetic persons. It is now known that chromium may potentiate the action of insulin, either by an effect on insulin-dependent functions, by maintaining these functions with lower insulin levels, or both. It is estimated that the minimum requirement in adults for Cr^{3+} is provided by a daily dietary intake of 2–8 μg; this amount corresponds to 0.03–0.13 μg Cr^{3+} kg^{-1} body wt. per day (Janus and Krajnc 1990). Glucose tolerance is usually altered in cases of total calorie and protein undernourishment (WHO 1988; Maria et al. 1999). The foods that contain chromium include oysters, calfs' liver, egg yolk, peanuts, grape juice, wheat germ, and black pepper. The average daily dietary intake from such foods is estimated to be 100 μg total chromium per day, with a range of 10–1,200 μg total chromium kg^{-1} body wt. per day (Emsely 1999). The upper limit of human intake for Cr^{3+} is 0.83 μg of absorbed-Cr^{3+} kg^{-1} body wt. per day (WHO 1988; Maria et al. 1999), whereas, the daily excretion rate has been measured to be in the range of 50–200 μg (Emsely 1999).

7 Bioremediation

Bioremediation is an attractive alternative pollution mitigation option that offers the possibility of using living entities to destroy or render harmless various contaminants. As such, bioremediation may be achieved at relatively low cost, may achieve high efficiency, and may utilize rather simple technological techniques. Moreover, bioremediation generally has high public acceptance, and can often be carried out on site. By definition, bioremediation is the use of living organisms, primarily microorganisms, to degrade or remove environmental contaminants and render them into nontoxic or less-toxic forms. This process uses naturally occurring bacteria, algae, and fungi or plants to degrade or detoxify substances that are hazardous to human health or to the environment. The microbes used in bioremediation may be indigenous to a contaminated area or they may be isolated from other sources and brought to a contaminated site. When microorganisms are incorporated onto a contaminated site to enhance remediation, the process is referred to as bioaugmentation (Vidali 2001). Microbial bioremediation offers an attractive treatment option, mainly because the technology is cost effective and environmentally compatible.

It has been revealed in several studies that microorganisms can interact with ions of heavy metals and radionuclides. Such interactions occur in many genera of microbes used for bioremediation, including *Bacillus*, *Enterobacter*, *Escherichia*, *Pseudomonas* species; moreover, some yeasts and molds are also useful in bioremediation of chromium-contaminated soil and water because they are capable of biosorbing and bioaccumulating chromium (James and Barlett 1983; Bopp and Ehrlich 1988; Wang et al. 1989; Ishibashi et al. 1990; Losi et al. 1994b; Shen and Wang 1994a; Cifuentes et al. 1996; Garbisu et al. 1998; Philip et al. 1998; Nies 1999; Kotas and Stasicka 2000). The biotransformation of Cr^{6+} to Cr^{3+} via microbial metabolism is well established as a pragmatic and feasible bioremediation approach to cleaning some contaminated sites. More recently, genetic engineering of cells to alter their morphological and physiological features has enhanced microbes in ways that may contribute to bioremediation efforts.

The use of nonexpensive waste biomass, the low cost of biomass immobilization, and the possibility of biomass regeneration are key factors that should be considered in selecting bioremediation as a strategy for removing toxic metals from wastewater (Gasbarro et al. 1997; Quintelas and Tavares 2001). Factors such as pH, temperature, chromium concentration, contact time, inoculum concentration, co-existence of other heavy metals, presence of carbon–nitrogen sources, and other kinetic parameters may affect the performance of Cr^{6+} bioremediation (see Fig. 2), and, hence, must be considered before choosing bioremediation as the preferred option at any particular site; such factors regulate the performance of applied remediation strategies in ecosystems that are polluted by either natural or anthropogenic hexavalent chromium.

There are four types of bioremediation strategies that are used to treat tannery waste. These include biosorption, bioaccumulation, bioreduction, and chromium removal by immobilized cells, and each of these is described in the following sections.

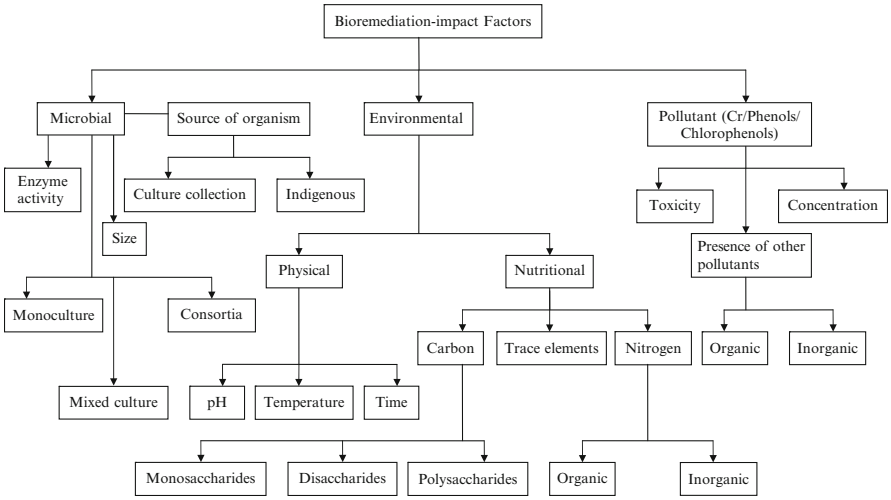

Fig. 2 Factors affecting the probable success of bioremediation of hexavalent chromium and phenolic/chlorophenolic compounds

7.1 Biosorption

Biosorption is the removal or recovery of free metal ions from a solution by a prokaryotic and/or eukaryotic biosorbent. The biosorption by microbes of hexavalent chromium under various optimized experimental conditions is shown in Table 1. Shumate and Strandberg (1985) defined biosorption as "a non-directed physicochemical interaction that may occur between metal/radionuclide species and the cellular components of biological species." Heavy metal ions can be entrapped in the cellular structure of such organisms and subsequently be biosorbed onto binding sites present on it. Biosorbents contain a variety of functional groups, including carboxyl, imadizole, sulfhydril, amino, phosphates, sulfate, thioether, phenol, carbonyl, amide, hydroxyl moieties, etc. Although most past and current research in this area is oriented toward removing cationic heavy metals, the biosorption of anions (like chromate) onto biomass has attracted growing interest in the field of biosorption (Kratochvil and Volesky 1998).

Biosorption can be mediated via both living and dead cell biomass. It is metabolism-dependent when living biomass is employed, and metabolism-independent in dead cells. The biosorption process always involves a solid phase which serves as the biosorbent (various biological materials). Due to higher affinity of the sorbent for the sorbate species, the sorbate is attracted and bound there by various mechanisms. The adsorption process continues until equilibrium is established between the sorbent and sorbate species concentrations and its residual quantity in the solution. The degree of sorbent affinity for the sorbate determines its distribution between the solid and liquid phases. This adsorption is based on mechanisms such as complexation, ion-exchange, coordination, adsorption, chelation, and microprecipitation, which

Table 1 Microbial biosorption of hexavalent chromium under various optimized experimental conditions

Microorganisms	Source of organism	Experimental conditions					Chromium removal		Reference
		System (medium/effluent)	Initial Cr^{6+} concentration (mg $L^{-1)}$	Initial biomass concentration (g L^{-1})	pH	Temperature (°C)	Time (h)	%	Uptake rate (mg g^{-1} bios)	
Bacillus coagulans	Tannery effluent	Shake flask (Cr^{6+} working solution)	100	2.0	2.5	28	1.0	–	39.9	Srinath et al. (2002a)
Bacillus megaterium			100	2.0	2.5	28	1.0	–	30.7	
Bacillus coagulans	Tannery effluent	Shake flask (Cr^{6+} working solution)	100	0.2	2.5	28	2.0	–	40.61	Srinath et al. (2003a)
Aspergillus oryzae	Culture collection (PTCC)	Shake flask (tannery effluent)	240 (Cr^{3+})	1.2	5.0	30	24	97.6	83.7	Sepehr et al. (2005)
Aspergillus niger MTCC 2594	Culture collection (MTCC)	Shake flask (spent chrome liquor)	383.7	2.0	3.8	28–32	36	78.7	3	Mala et al. (2006)
Aspergillus sp.	Tannery effluent	Shake flask (MSM)	500	100	6.0	30	168 (7 days)	88	12.9	Srivastava and Thakur (2006)
Saccharomyces cerevisiae	Fermenter brewing beer at an industry	Shake flask (tannery effluent)	1230	10	7.0	28–30 (ambient)	1.0	99.1	122	Parvathi et al. (2007)
Bacillus coagulans	Culture collection (STCC)	Mini-column (synthetic medium)	100	–	7.2	37	72	–	5.35	Quintelas et al. (2008)
Escherichia coli			100	–	7.2	37	72	–	4.12	
Streptococcus equisimilis			100	–	7.4	37	72	–	5.82	

Arthrobacter viscosus	Culture collection (STCC)	Shake flask (Cr^{6+} working solution)	100	5.0	4.0	28	1752 (73 days)	72.5	12.6	Silva et al. (2009)
Pseudomonas aeruginosa + *Bacillus subtilis*	Culture collection (NCIM)	Shake flask (Cr^{6+} working solution)	10	0.5	2.0	32	0.38 (23 min)	–	1.44	Tarangini et al. (2009)
Yeast biomass	Natural habitat	Shake flask (Cr^{6+} working solution)	120	1.0	4.0	30	0.75 (45 min)	95	86.95	Lokeshwari and Joshi (2009)

may occur either independently or synergistically (Mejare and Bulow 2001; Ahalya et al. 2003). Various biomaterials, including microbial cells, have been identified and documented as effective metal-removing agents (Volesky and Holan 1995; Veglio and Beolchini 1997). The biosorption process has certain distinct advantages over conventional methods (Kratochvil and Volesky 1998). First, the process does not produce chemical sludge, and it can be highly selective, more efficient, easy to operate, and hence cost effective for treating large volumes of wastewaters that have low metal concentrations. Second, the biomass is not affected by other toxic chemicals, metals, and unfavorable factors that are generally associated with effluents. Third, the biosorbent can be regenerated and reused several times (Gadd 1990), and fourth, the biosorption process has short operation times (Brierley et al. 1986).

Bacteria are highly competent (Beveridge 1988) and efficient (Kurek et al. 1982; Walker et al. 1989) adsorbents as a result of their high surface area-to-volume ratio. The additional qualities that bacteria have as biosorbents are their ubiquity, ability to grow under controlled conditions, and their resilience to a wide range of environmental situations (Urrutia 1997; Quintelas and Tavares 2001). The strong biosorbent behavior of certain types of microbes toward metallic ions is a function of the chemical makeup of their cells. The crucial aspects of an efficient biosorption process are localization of metal deposition sites within the biosorbent biomass, understanding the metal-sequestering mechanism, elucidation of the relevant metal solution chemistry, and chemical structure of the metal deposition site.

Metals biosorb to microbial cells via three different processes: (1) extracellular accumulation/precipitation, (2) cell surface sorption/precipitation, and/or (3) intracellular accumulation. The last type of biosorption requires transport of the metal across the cell membrane, hence it is metabolism-dependent, and can be performed only by viable cells. Although metabolism-independent biosorption is a passive phenomenon, it depends totally on the physicochemical properties of the cell wall and its architecture (Langely and Beveridge 1999), which varies phenotypically and depends on the growth media used (Wong and So 1993; Simmons and Singleton 1996; Andres et al. 2000), and on the metabolic state of the cells (Donocik et al. 1996; Andres et al. 2000). Classical studies on the biosorption of metals by isolated cell walls of *Bacillus subtilis* (Beveridge 1978) suggested that, during biosorption, metal molecules first complex with existing reactive sites on cell wall polymers, then additional metal crystallizes on these bound metals. This type of metabolism-independent biosorption is relatively rapid and can be reversible (Kuyucak and Volesky 1988). The biosorption phenomenon is more or less like a chemical reaction, and thus, several parameters affect the process. These factors are nature and structure of microbial layers, physiological state of the cell, pH of the medium, contact time, microbial growth conditions, temperature, ionic speciation, concentration of biomass and concerned metal, etc. The succeeding paragraphs dwell on some of these intricate features.

7.1.1 Biosorption by Living vs. Dead Cell Biomass

Dead cell biomass is much better than living cell biomass at biosorbing heavy metals. The efficiency for biosorption increases as live cells are ruptured after heat treatment,

because such treatment yields cells that have more binding sites. The increased biosorption performance of dead cells over living ones occurred with *Myxococcus xanthus* and *Saccharomyces cerevisiae* for numerous heavy metals, including chromium (Omar et al. 1997). Dead cells of *M. xanthus* and *S. cerevisiae* biosorbed Cr^{6+} at approximate rates of 0.27 mM (14 mg g^{-1}) and 0.22 mM (11.4 mg g^{-1}), which was, respectively, 7.0% and 5.3% higher than the biosorption rates of living cells. Similarly, Srinath et al. (2002a) observed Cr^{6+} biosorption to significantly increase from 23.8 in live cells to 39.9 mg g^{-1} in dead ones of *Bacillus coagulan*s. This performance difference can be attributed to a pH change between live and dead cells, which is the most important factor affecting biosorption (Paknikar et al. 1999).

The uptake of Cr^{6+} is governed by an "acid adsorption" mechanism, wherein the liquid should have enough protons to cause anion exchange (Sharma and Forster 1993; Kratochvil et al. 1998). When living cells are used for metal biosorption in an unbuffered condition, the redox reactions between the cells and liquid causes an increase in final pH, which hampers maximal uptake. In contrast, the equilibrium pH remains relatively unaltered in dead biomass, which is attributed to the prior conditioning of the dead biomass to the desired pH. Therefore, in dead cells the maximal binding sites remain available for Cr^{6+} biosorption (Srinath et al. 2002a). Park et al. (2005) reported that the dead biomass of four fungal strains *Aspergillus niger*, *Rhizopus oryzae*, *S. cerevisiae*, and *Penicillium chrysosporium* completely removed the initial 50 mg Cr^{6+} L^{-1} concentration from aqueous solution. *R. oryzae* was more effective, and completely removed Cr^{6+} in 48 h, whereas others required periods ranging between 218 and 254 h. The removal rate of Cr^{6+} increased as pH decreased, and as Cr^{6+} and biomass concentrations increased. The maximum biosorption that occurred with *Pseudomonas* sp. at 100 ppm initial Cr^{6+} concentration was 44% and 49.6% in 240 min by live and dead biomass, respectively (Murugesan and Maheswari 2007). Tripathi et al. (2011a) also reported that the biosorption performance of *Bacillus cereus* was 15.8% higher in dead cell biomass than in live cell biomass. In contrast, Faisal and Hasnain (2004) reported an 8.3% and 41.3% higher Cr^{6+} uptake by living biomass as compared to dried and heat-killed cell biomass, respectively, at an initial 1,000 mg Cr^{6+} L^{-1} concentration.

7.1.2 Hydrogen Ion Concentration (pH)

The pH of the metal solution is one of the most influential factors affecting the surface properties of biomass and metal speciation (Paknikar et al. 1999). The uptake of metallic cations by cells/biomass is reduced at a pH of 2.0 and above a pH of 8.0; uptake is lowest for metallic cations at their isoelectric point. Cr^{6+} is present as dichromate ($Cr_2O_7^-$) in acidic environments (pH 3–6) and as chromate ($Cr_2O_4^-$) in alkaline environments, when the pH is >8.0. Numerous investigators have reported that the pH range from 4.0 to 8.0 is optimal for uptake of cations by the negatively charged surface of bacteria (Tobin et al. 1984; Volesky and Holan 1995; Kratochvil et al. 1998). However, oxyanions, like chromate, are sorbed favorably under acidic conditions (Sharma and Forster 1994a, b: Kratochvil et al. 1998; Srinath et al. 2002a). This pH specificity renders Cr^{6+} selectively recoverable from solutions containing

an amalgam of heavy metal cations and anions (Volesky 1990). The high adsorption of Cr^{6+} at low pH varies with chromium (chromate) species and adsorbent surface characteristics. Bacterial cell walls are negatively charged under acidic pH conditions. The chemical functional groups of cell walls display high affinity for metal ions in solution (Collins and Stotzky 1992). The surfaces of adsorbents become highly protonated, and favor the uptake of Cr^{6+} in anionic form under acidic conditions. The degree of protonation, and hence, the adsorption decreases with an increase in pH. Furthermore, there is competition between hydroxyl and chromate ions for binding; the former is the dominant species at higher pH values. Moreover, as the pH increases, the net positive charge on the surface of sorbents decreases from reduced protonation, which ultimately leads to reduced sorption capacity.

Srinath et al. (2003b) reported enhanced Cr^{6+} biosorption by *B. coagulans* as the pH decreased from 5.5 to 2.5 (maximal adsorption occurred at pH 2.5). Further decrease in solution pH from 2.5 to 1.0 reduced the chromium uptake. It is suggested that at a lower pH, $HCrO_4^-$ ions start oxidizing the biomass, thereby producing Cr^{3+} ions, which then compete with protons via cation exchange reactions for binding sites on the biomass (Kratochvil et al. 1998). Thus, the optimum pH for Cr^{6+} biosorption occurs when the reduction/oxidation potential of $HCrO_4^-$ equilibrates. Further, although the surface of *Bacillus* sp. is cationic in nature, the anionic moieties are evenly dispersed throughout the cell wall. Therefore, at an acidic pH, the surface charge on *Bacillus* sp. is favorable for anionic metal adsorption (Niu and Volesky 1999). At an initial concentration of 200 mg Cr^{6+} L^{-1} in the presence of pentachlorophenol (500 mg L^{-1}), Tripathi et al. (2011a) reported a maximum Cr^{6+} biosorption of 35.2 ± 1.89 and 42.5 ± 1.28 mg Cr^{6+} g^{-1} dry wt. of live and dead cells at pH 5.0 and 4.0, respectively by *B. cereus*. The authors observed that any deviation from the initial pH 5.0 resulted in reduced biosorption by live bacterial cells. Furthermore, when live biomass was employed for Cr^{6+} biosorption in unbuffered condition, the redox reaction between the cells and liquid caused a decrease in the final pH, regardless of the initial pH values under study. In contrast, the equilibrium pH was relatively unaltered for dead biomass that had prior pH conditioning to a desired pH of 4.0 (based on availability of maximum binding sites for Cr^{6+} biosorption). A similar behavior of Cr^{6+} biosorption was observed in several nonmicrobial biosorbents; these included *Sargassum* sp., peat moss, sugarcane bagasse, beet pulp, maize cob, activated carbon, among others (Huang and Wu 1977; Sharma and Forster 1993, 1994a, b; Kratochvil et al. 1998). Parvathi et al. (2007) reported a neutral pH optimum for the maximum Cr^{6+} biosorption efficiency (99%) of dead *S. cerevisiae* biomass (from 1230 mg L^{-1} Cr-laden raw tannery effluent).

7.1.3 Temperature

The mechanism by which dead cell biomass biosorbs metals is metabolism-independent. Therefore, it is less likely to be affected by temperature, since the processes responsible for removal are largely physicochemical in nature (Gulay et al. 2003). Still, chromate biosorption by *Rhizopus arrhizus* (Sag and Kustal 1996a, b)

and *Zoogloea ramigera* (Sag and Kustal 1989) was affected by temperature; however, the effect was less for *B. coagulans* over a rather broad range (20–40°C) (Srinath et al. 2003b). A broad temperature range (25–40°C) for Cr^{6+} biosorption by *B. cereus* has also been reported by Tripathi et al. (2011a). With an increase in temperature from 25°C to 35°C, there was only a marginal increase in Cr^{6+} biosorption. A further increase in temperature to 40°C caused a slight decrease in Cr^{6+} biosorption. Since a temperature of 25–35°C is the most prevalent range that occurs in tropical countries, the biosorption of Cr^{6+} by *B. coagulans* and *B. cereus* will tend to be independent of temperature, because the maximum sorption by both species is in the same temperature range.

Any enhancement of metal sorption that occurs can be attributed to increased energy in the system that facilitates metal attachment to cell surfaces. A decrease in metal sorption may also result from distortion of cell surface sites (Al-Asheh and Duvnjak 1995). It is suggested that an increase in metal uptake at higher temperatures may result either from a higher affinity of sites for the metal, or from an increase in the biomass binding sites (Marques et al. 1991).

7.1.4 Presence of Other Metal Ions

Natural habitats are generally characterized by the coexistence of many toxic and nontoxic cations and anions. Similarly, industrial effluents rarely or never have only single metal or pollutant species. Effluents generally contain a blend of numerous cations and anions of metals and nonmetals, the latter of which may either hinder or assist in binding of the concerned metals. Therefore, when studying bioremediation strategies, it is necessary to evaluate the effect of metal/pollutant mixtures present on the growth rates of microorganisms (Verma and Singh 1995). When microbes are used in bioremediation, it is an advantage for them to have tolerance to metals other than the targeted ones, so that they can withstand their presence in effluents as they perform their desired bioremediation activities. The advantage of selecting indigenous microbes from contaminated environments is that they may, through the development of natural resistance, minimize the inhibitory effects that result from the presence of constituents other than the targeted Cr^{6+}. Furthermore, it is practical to use Cr^{6+}-reducing microorganisms that can concomitantly remove other metals also (Lovely 1995). Tripathi et al. (2011b) reported the presence of other metal ions in treated tannery effluent. Depending on the degree of their competitiveness and toxicity, the presence of such ions can complicate the biosorption process (Volesky and Holan 1995).

Biosorption of Cr^{6+} by *B. coagulans* biomass was unaffected by the presence of chloride ions, probably because of the dissimilarity of their ionic feature. Sulfate at 200 mg L^{-1} competitively inhibited the biosorption of Cr^{6+} by 16%, which probably resulted from the similarity of its ionic features to chromate (Srinath et al. 2003b). Cells of *Chlorella vulgaris* biosorbed Cr^{6+} and copper optimally at different pH (pH 2.0 and 4.0) but the biosorption of these metals was competitive (Aksu and Acikel 1999). The biosorption of Cr^{6+} and Fe^{3+} was antagonistic for the cells of *R. arrhizus*

(Sag et al. 1998). At a pH of 5.0, *Zoogloea* biosorbed Cu, Cd, and Zn selectively, while chromium was not biosorbed, demonstrating that biosorption of a metal can be selective, and can vary according to species and even strain (Ahn et al. 1998).

Srinath et al. (2003b) studied the effect of various heavy metals on biosorption of Cr^{6+} by *B. coagulans* at a pH of 2.5. These authors observed no enhancement in Cr^{6+} uptake. Lead (Pb), at levels up to 150 mg L^{-1}, did not affect Cr^{6+} uptake. An 11.8% decrease in Cr^{6+} biosorption was noted in the presence of a solution of 200 mg Pb L^{-1}. When multimetals were present, Cr^{6+} biosorption decreased. Although molybdate has similar ionic features, the optimal pH of 2.5 for Cr^{6+} rendered its biosorption more selective (Srinath et al. 2003b). Furthermore, the solubility of molybdate at an acidic pH is minimal, because it loses anionic features as a result of its protonation at low pH (Zhao et al. 1996). Similarly, Tripathi et al. (2011a) found that several cations produced an insignificant effect on the biosorption of Cr^{6+} by *B. cereus*. The reason for this is explained by the theory of acid–base equilibrium. In the pH range of 2.5–5.0, the degree of binding of cationic heavy metals is determined primarily by the dissociation state of the weakly acidic groups. Furthermore, competition for metal binding sites may exist between metal ions and hydrogen (H^+) and hydronium (H_3O^+) ions (Gadd 1988; Antuner et al. 2001); in addition, electrostatic repulsive force may exist between the protonated biomass and cations present.

7.1.5 Initial Biosorbent and Metal Concentration

The initial Cr^{6+} and biosorbent concentrations are important in the effectiveness of the biosorption process. The initial ratio of the metal ions to biosorbent concentration plays a decisive role during the initial treatment process optimization. At a given equilibrium metal concentration, biomass adsorbs more metal ions at lower cell densities (Sharma and Forster 1994a; Paknikar et al. 1999). Srinath et al. (2003b) reported a decrease in Cr^{6+} uptake, with an increase in *B. coagulans* biomass concentration from 2 to10 g L^{-1}. A curvilinear adsorption isotherm for Cr^{6+} biosorption was obtained, which revealed that metal uptake was chemically equilibrated in a manner that involved a saturable mechanism. Thus, there was an increase in metal uptake as long as the binding sites were free. Other researchers (Sharma and Forster 1994b; Merrin et al. 1998) also made similar observations on the biosorption of Cr^{6+}. Tripathi et al. (2011a) observed that the optimum *B. cereus* biosorbent dose for maximum Cr^{6+} biosorption was 2 g L^{-1}. At the optimum biosorbent level, a sufficiently large number of binding sites were available on the biomass for electrostatic interaction. At higher biosorbent concentrations, the metal-to-microbe ratio (Puranik and Paknikar 1999) decreased and the metal uptake value marginally decreased; this produced excessive free extra binding sites that were available on the biosorbent, which may have decreased the electrostatic interaction between metal and binding sites of the biosorbent (Fourest and Roux 1992).

Faisal and Hasnain (2004) reported that the Cr^{6+} uptake by bacterial strains (CrT-11, CrT-12, *Bravibacterium* sp., CrT-13, and CrT-14) increased with an increase in $K_2Cr_2O_7$ concentration from 100 to 1,000 μg mL^{-1}, and with contact time; the maximum

uptake occurred at 4 h incubation. Quintelas et al. (2006) reported a decrease in percent removal of Cr^{6+} at initial higher metal concentrations by living cells of *Arthrobacter viscosus* biofilm, owing to the toxicity and high oxidation potential of chromate. Tripathi et al. (2011a) performed biosorption experiments at initial Cr^{6+} concentrations that ranged from 50 to 250 mg L^{-1}, and an optimized fixed dose (2 g L^{-1}) that employed live and dead *B. cereus* biomass. As the initial Cr^{6+} concentration increased from 50 to 250 mg L^{-1}, the percentage of Cr^{6+} removed from solution decreased from 97.5% to 57.0% and 98.5–59% for live and dead cells, respectively. At lower concentrations (<200 mg Cr^{6+} L^{-1}), metal ions in the solution possibly interacted with the binding sites, and thus facilitated a higher removal of Cr^{6+}. In contrast, at higher concentrations, more chromium ions were left unadsorbed in the solution from the increased metal levels that were competing for biosorbent binding sites. Hence, the percentage of chromium removed was dependent on the initial metal concentration (Puranik and Paknikar 1999). Moreover, at higher Cr^{6+} concentrations, the average distance between adsorbing species also decreased (Horsfall et al. 2006). Thus, for such experiments it is essential to determine the optimum initial ratio of the biomass to metal concentration (Puranik and Paknikar 1999).

7.1.6 Contact Time

For attaining equilibrium, knowing the duration of contact between the biomass and the metal ions is relevant to how a process will behave. Such information is also essential for economical industrial exploitation. Biosorption occurs rapidly, if equilibrium is optimally attained within a few hours. At a pH of 2.0, the biosorption of Cr^{6+} by *R. arrhizus* was rapidly achieved (i.e., 82% adsorption occurring within 1 h, and 92.5% after 8 h; Merrin et al. 1998). Further incubation did not enhance the adsorption of Cr^{6+}. In contrast, Cr^{6+} biosorption by other strains of *R. arrhizus* exhibited a slower biosorption rate (Sag and Kustal 1996a, b; Prakasham et al. 1999). Srinath et al. (2003b) reported that biosorption of Cr^{6+} by *B. coagulans* biomass occurred rapidly, attaining equilibrium within 60 min; further incubation (240 min) increased biosorption by only 4%.

Various steps occur as a metal transfers from bulk solution to binding sites on the biomass (Weber 1985). The first step is rapid, because of mixing and advective flow (Gadd 1988). The second step is film transport involving diffusion of the metal through a thermodynamic boundary layer around the biosorbent surface. The third step is actual adsorption of the metal ions by active sites on the biomass. This step is rapid, and is equivalent to an equilibrium reaction (Weber 1985). In the case of Cr^{6+} biosorption by *B. coagulans*, experimental conditions allowing good mixing of solutes and biomass may have suppressed kinetic limitation in the first and second steps. In the third step, the rate of Cr^{6+} biosorption is influenced by the transfer of metal from solution to biomass binding sites (Srinath et al. 2003b). Sau et al. (2008) reported that the quantity of chromium adsorbed by dry and wet dead cell mass of *Bacillus firmus* increased as contact time increased. In addition, dried cell mass adsorbed a somewhat higher quantity of chromium than did dead wet cell mass.

Tripathi et al. (2011a) studied the effect of contact time on biosorption of Cr^{6+} under optimum conditions. The process they studied attained equilibrium at 120 min (70.5% Cr^{6+} removal), and remained nearly constant as the contact time was increased to 140 min.

7.1.7 Role of Exopolysaccharides in the Biosorption Process

Many microbial polysaccharides are known to bind heavy metals, and by so doing display varying degrees of specificity and affinity. Exopolysaccharides (also called extracellular polysaccharides or EPS) comprise a complex mixture of polysaccharides and proteins (Omoike and Chorover 2004), lipids or humic substances (van Hullebusch et al. 2003). These EPS building molecules contain ionizable functional groups (e.g., carboxyl, phosphoric, amine and hydroxyl, etc.) (van Hullebusch et al. 2003). EPS have several important microbial functions, including fomenting adhesion to surfaces, aggregating bacterial cells in flocs and stabilizing them, forming a protective barrier that provides resistance to biocides or other harmful entities, retention of water, sorption of exogenous nutrients, and uptake of metals (Comte et al. 2006). Some of these functions play a key role in metal biosorption processes. The EPS produced by various microorganisms, such as algae, bacteria, and fungi, are recognized as being potentially useful in meeting the need for economical, effective, and safe disposal methods of heavy metals from wastewater. Two steps are required for attaching microbes onto solid surfaces to form biofilms. First, microorganisms approach solid surfaces via weak electrostatic forces. Second, these microbes produce EPS, which eventually form a biofilm matrix that remains firmly adhered to its solid support (Parkar et al. 2001). The presence of binding sites enables EPS to both sequester minerals and nutrients for microbial growth, and also to remove toxic metals during biological treatment of wastewater (Liu et al. 2002).

Unfortunately, few reports exist on microbial metal chelation via an EPS mechanism. The exopolymer referred to as the capsule/slime layer in some microbes (e.g., *Z. ramigera*, *Klebsiella aerogenes*, *A. viscosus*, and *Pseudomonas* sp.) constitutes the major cellular component that performs biosorption (Volesky 1990; Gadd and White 1993). Iyer et al. (2004) reported that EPS of *Enterobacter cloaceae* have high incidence of uronic acid and sulfate groups, which may be responsible for chelation, because these organisms successfully chelated ~75% of a solution having an initial 100 ppm chromium concentration. The authors observed that Cr^{6+} stimulated the production of polysaccharides in a concentration-dependent manner. Quintelas et al. (2008) studied three different polysaccharide-producing bacterial species (*Streptococcus equisimilis*, *B. coagulans*, and *E. coli*) that were supported on granulated activated carbon, for their ability to remove Cr^{6+} from either aqueous batch or column solutions. The subsequent quantification of polysaccharides (that play a key role in the whole process of chromium adsorption) revealed that their production was highest in *B. coagulans,* followed by *S. equisimilis* and then *E. coli* (9.19, 7.24, and 4.77 mg g^{-1} biomass, respectively). All bacteria had good adhesion to granular activated carbon and good qualities for chromium ion entrapment.

7.1.8 Effect of Chemical Pretreatment of Biosorbent-Biomass

The biosorption potential of a biosorbent can be altered by chemical pretreatment of the biomass (Akthar et al. 1995; Puranik and Paknikar 1999; Srinath et al. 2002a). The alteration may result from cleansing effects and modification of binding sites, changes in the overall surface charge, masking/unmasking of binding sites and/or more complex actions such as formation of electrostatic bonds. Siegel et al. (1986) reported that biomass that lost Cr^{6+} biosorption capability, regained it when the biosorbent was pretreated with $Pb(NO_3)_2$. It was suggested that the Pb, which was preloaded on the biomass, reacted and precipitated as lead chromate. Parvathi et al. (2007) treated *S. cerevisiae* biomass with NaOH at pH 7.0, 9.0, and 11.0. This biomass then exhibited significant biosorption capacity as compared to raw and HCHO–HCOOH treated biomass. At pH 4.0, untreated biomass exhibited maximum chromium sorption, compared to biomass treated with NaOH and HCHO–HCOOH. The authors asserted that pH affected not only the biosorption capacity of raw biomass, but also the behavior of the pretreated biomass. Methylation of amines by HCHO–HCOOH pretreatment prevented the participation of biomass amino groups in metal binding at pH of 4.0, 7.0, and 11.0. However, at pH 2.0, an opposite trend was evident, due to an unmasking of some cellular groups, which were unable to participate in biosorption without the acid–aldehyde treatment. To summarize, pretreating biomass with NaOH increased the sorption of Cr^{6+}, when the pH was between 4.0 and 11.0; but, at pH 2.0, the sorption increase was much more modest.

7.2 Bioaccumulation

Bioaccumulation occurs by several processes, all of which result in the uptake of bioavailable metal ions by living cells. Such processes include adsorption, intracellular accumulation, and bioprecipitation. Certain heavy metals play important roles as “trace elements,” and these are key to biochemical growth and maintenance (Huges and Poole 1989). Of 22 heavy metals, 17 have biological functions under some physiological conditions (Nies 1999). Among these, Fe, Mo, and Mn are important trace metals that have low toxicity. Others, like Zn, Ni, Cu, V, Co, W, and Cr, are toxic elements that have high to moderate importance as trace elements. Other heavy metals such as As, Ag, Sb, Cd, Hg, Pb, and U have limited beneficial functions, and are considered to be toxic. Among living organisms, bacteria are the first to interact with metals in the environment. Consequently, living organisms have evolved mechanisms for the active transport and/or extrusion of these metal ions, thereby enabling cells to regulate intracellular concentrations by relying on a family of metal-ion transport proteins (Paulsen and Saier 1997).

Microbial heavy metal accumulation often occurs in two stages (Failla 1977; Trevors et al. 1986; Belliveau et al. 1987; Gadd 1988, 1990). First, an initial rapid and passive process occurs in which metals are physically adsorbed or ions exchanged at cell surfaces. Second, a slower phase occurs, that involves active

metabolism-dependent transport into bacterial cells. This second phase is inhibited by low temperatures, by the absence of an energy source, and by metabolic inhibitors and uncouplers. As occurs for other substances in phase two, the bioaccumulation process for metals occurs via a metabolism-dependent mechanism. The rate of uptake is also influenced by the state of cells and the composition of the medium in which they exist (Gadd 1990), and can also be affected by the initial metal concentration (Srinath et al. 2002b). Most metal transport mechanisms rely on the electrochemical proton gradient across the cell membrane. Such gradients have a chemical component (pH gradient), and an electrochemical gradient (the membrane potential) component. Each can drive the transport of ionized solutes across the membrane. Specific uptake systems do not exist for chromates, but in the *Ralstonia* sp. strain CH34, chromate enters via the sulfate-uptake system (Nies 1999), which possibly results from the similitude of their three-dimensional structures.

Hydrogen ion concentration (i.e., pH) is an important index that can reflect microbial activity. The pH of a solution is known to modulate ion speciation, cellular metabolism, and sites of interactions that may produce changes in both the accumulation and toxicity of metals (Volesky 1990; Beveridge and Graham 1991). The extent to which Cr^{6+} is accumulated by *Bacillus circulans* bears relation to the initial pH of the medium in which they exist. Srinath et al. (2002b) studied this relationship at pH from 4.0 to 8.0. Cr^{6+} accumulation was optimal and equal between pH of 6 and 8. At a pH of 5.0, Cr^{6+} accumulation was significantly reduced, and at pH 4.0, it was negligible. Whether Cr^{6+} was present or absent, after 24 h of growth at an initial pH of 6–8, the final pH approached 7.7. At an initial pH of 5.0, the final pH of the medium of the unchallenged *B. circulans* cells was 6.3, whereas the final pH in the presence of Cr^{6+} was 5.5. This alteration illustrated what effect pH has on the cellular metabolism and toxicity of Cr^{6+}. Srivastava and Thakur (2007) studied the effect of pH on chromium bioaccumulation in *Acinetobacter* sp., and reported that 85–89% of the chromium was removed (from initial 500 ppm Cr^{6+} concentration); the uptake rate was 12.3–13.5 mg g^{-1} dry wt. of cell biomass during the 7-day incubation period. In a 2-L laboratory scale bioreactor (flow rate 20.8 mL h^{-1}), tannery effluent containing 557 ppm Cr^{6+} was supplemented with optimized 0.2% (w/v) sodium acetate and 0.1% (w/v) sodium nitrate, the pH of which was maintained at 7.0–7.5, and then was inoculated with *Acinetobacter* sp. The amount of chromium removed was 80% (112 ppm), and the rate of uptake was 19 mg g^{-1} dry wt. at the 15th day. Congeevaram et al. (2007) isolated chromium-resistant microorganisms (*Aspergillus* sp. and *Micrococcus* sp.) from a heavy metal-contaminated environment. The suitability of using these isolates as means of chromium removal from industrial wastewater was evaluated at different pH ranges. The maximal Cr^{6+} removal (i.e., 92% and 90%) occurred at pH 5.0 and 7.0 by *Aspergillus* sp. and *Micrococcus* sp., respectively; the initial level was 100 mg Cr^{6+} L^{-1} and incubation period was 24 h. The effect of pH that was observed on bioaccumulation of chromium was attributed mainly to organism-specific physiology.

Temperature affects the rate of Cr^{6+} bioaccumulation by affecting the stability of the microbial cell wall, its configuration, and rate of ionization of chemical moieties (Volesky 1990). Temperature may act synergistically with other factors to affect

bacterial cell binding sites, thereby changing chromium bioaccumulation rates. The optimal temperature for reduction of Cr^{6+} generally coincides closely with the conditions that are optimal for cell growth. Besides the influence of initial pH, Srinath et al. (2002b) also studied the effect of temperature (20–40°C) on bioaccumulation of Cr^{6+} by *B. circulans,* during a 24-h incubation, and reported no significant temperature effects in the range of 25–35°C. The authors reported that any deviation from the optimum range (25–35°C) reduced chromium accumulation significantly. Further, the bioaccumulation of Cr^{6+} by *B. circulans* was growth mediated. Since there was no growth at 45°C, no accumulation of Cr^{6+} occurred. Parameswari et al. (2009) studied Cr^{6+} removal efficiencies of *Azotobacter chroococcum*, *Bacillus* sp., and *Pseudomonas fluorescens* that were isolated from sewage environment. They observed a maximum removal of 82.58%, 89.50%, and 95.54% chromium, respectively for these three species at 35°C over the 72-h incubation.

Because temperature is an important factor for bacterial growth, it will necessarily affect enzymes that may be essential for chromium removal. At temperatures above the optimum, cell function declines and growth processes slow or fail. At suboptimal temperatures, metabolic regulation will be affected and may fail. Hence, only at optimum temperatures do bacteria appropriately utilize their substrates, providing other optima for environment, nutrition, etc. are met.

Srinath et al. (2002b) studied the effect of continuous exposure of *Bacillus circulans* cells to Cr^{6+} on growth and metal uptake. The difference in population density attained by Cr-exposed cells in the presence and absence of Cr^{6+} was insignificant. However, the exposed cells attained their maximum density in a shorter time span. In addition, accumulation of chromium by the Cr^{6+}-induced cells started slightly earlier than untreated cells, although there was no significant difference in accumulated Cr^{6+} content.

The initial concentration of metal ions can have a profound effect on the rate and quantity of metal that is bioaccumulated. Fude et al. (1994) reported a decreased rate of Cr^{6+} bioaccumulation as the initial concentration of chromium increased. Similarly, Srinath et al. (2002b) observed that chromium accumulation and the rate of chromium uptake were both dependent on the initial Cr^{6+} concentration, and a linear relationship between the two was established. The rates of Cr^{6+} accumulation by *B. circulans* were 19.55, 16.99, and 14.05 ($\times 10^2$ h^{-1}) for 25, 50, and 100 mg Cr^{6+} L^{-1}, respectively. Moreover, after equilibrium was attained, there was a slow enhancement in chromium uptake. Srivastava et al. (2008) reported the effect of initial chromium concentration (1.0–4.0 mg L^{-1}) on chromium bioaccumulation in *Pseudomonas* sp., during a 72-h incubation. They observed increased rates of chromium removal (i.e., 0.873, 1.840, 2.78, and 3.502 mg L^{-1}, respectively) as the initial Cr^{6+} concentrations of 1.0, 2.0, 3.0, and 4.0 mg L^{-1} were increased. Polti et al. (2011) reported that the *Streptomyces* sp. MC1 was able to accumulate up to 3.54 mg Cr^{3+} g^{-1} wet biomass, and removed 13.9% chromium from the culture medium, when the initial concentration was 50 mg Cr^{6+} L^{-1}. The authors observed that the chromium removed by *Streptomyces* sp. accounted for the intracellular accumulation.

Other heavy metals (cations and anions) commonly present in effluents have variable effect(s) on the bioaccumulation of Cr^{6+}, and how much of an effect they

have depends upon the type and concentration of ions present. Srinath et al. (2002b) performed binary metal studies, and observed that chromium bioaccumulation by *B. circulans* was dependent on the concentration of other metals used to challenge Cr^{6+}. At 25 mg cation(s) L^{-1}, Cr^{6+} accumulation efficiency exceeded 85%. Nickel at 100 mg L^{-1} was not tolerated by *B. circulans*. But interestingly, lead affected Cr^{6+} accumulation the least, and was tolerated the most. This may make sense as lead would have enabled Cr^{6+} to be accumulated in the form of lead chromate (Siegel et al. 1986). At pH of 6.0–8.0, the negative charge on a bacterial surface is optimal, so that the maximum of binding sites are offered for interaction of cations (Volesky 1990; Gadd and White 1993; Volesky and Holan 1995). Such cation interactions may physically or metabolically hamper Cr^{6+} uptake. Guan et al. (1993a, b) suggested that accumulated Cr^{6+} may act as a terminal electron acceptor, and the reduced Cr^{3+} then binds to the cell wall. Hence, cations present in the medium may reduce the accumulation efficiency by showing greater affinity for the Cr^{3+} binding sites. Some cations like Zn and Cu are trace elements (Nies 1999) that affect Cr^{6+} uptake at concentrations >50 mg L^{-1}. In multimetal (Cd, Cu, Pb, Ni, Zn) solution studies, Cr^{6+} removal efficiency decreased from 100% to 57.1% at 25 and 50 mg cations L^{-1}, respectively (Srinath et al. 2002b).

The effect of various anions on the accumulation of Cr^{6+} by *B. circulans* was also studied by Srinath et al. (2002b). They noted no enhancement in Cr^{6+} bioaccumulation efficiency. Rather, sulfate and molybdate ions significantly reduced the level of Cr^{6+} bioaccumulation. It is known that the sulfate-permeate system is used by Cr^{6+} for entry into cells (Kimbrough et al. 1999; Nies 1999), and thus has a role in competing at the sites for uptake. Chromate availability to cells may be limited by sulfate ions, which competitively inhibit the uptake of chromium (Ohtake et al. 1990a; Silver and Walderhaug 1992). Similarly, molybdate also utilizes the same ionic port as does sulfate, however, less competitively than chromate. Srinath et al. (2002b) further observed that chloride interfered with Cr^{6+} uptake the least. A mixture of these cations significantly reduced (~20%) the bioaccumulation of Cr^{6+}, at 100 mg L^{-1} concentration of the anions.

Bioaccumulation is an active process, and therefore, is affected by the physiological state of the cells. Cells of a denitrifying bacterial consortium that were starved for 50 days showed 10–15% higher levels of Cr^{6+} bioaccumulation (Kong et al. 1992, 1993) than did unstarved forms of these cells. A higher concentration of Cr^{6+} existed in the cell wall fraction from starved cells vs. fresh cells (Kong et al. 1992). This may have been caused by a reduction of Cr^{6+} to Cr^{3+} in the cell wall (Volesky 1990). In fresh cells, maximum amount of chromium was present in the cells' soluble fraction. However, in the presence of sulfate ions, Cr^{6+} bioaccumulation was reduced by half in both fresh and starved cells (Kong et al. 1993). The soluble fraction of *Pseudomonas aeruginosa* G-1 cells also contained the highest amount of chromium (Horitsu et al. 1983). Intracellular sequestration (compartmentalization) by *P. syringae* (Cha and Cooksey 1991) and *P. aeruginosa* (Kazy et al. 1999), as occurs with Cu^{2+}, did not occur with Cr^{6+}. However, extracellular precipitation of chromium was reported to take place in *Thiobacillus ferrooxidans* DSM 583 (Baillet et al. 1998); this strain bioaccumulated 501 mg Cr mg^{-1} dry wt. when held at a pH

of 2.4. Cr precipitated mainly at the outer cells surface, owing to the positive charge of the cells (Kar and Dasgupta 1996).

A consortium of sulfate-reducing bacteria (SRB-III), isolated from an electroplating effluent, tolerated a concentration of Cr^{6+} up to 2,500 mg L^{-1} (Fude et al. 1994). The authors observed that UV-killed cells did not accumulate significant amounts of chromium, whereas the uptake of Cr^{6+} by living cells was significantly hampered in the presence of molybdate. From these observations, the authors ascertained that uptake of Cr^{6+} was dependent on cellular metabolism, and the sulfate port was utilized for it. After 80 h of incubation, a >94% Cr^{6+} removal was attained from the initial 50–1,000 mg L^{-1} concentrations. In the presence of Zn (at 100 ppm) and U (at 50 ppm), a 5.1% increase in Cr^{6+} removal occurred, but at higher Cr concentrations (>1,000 ppm), precipitation of chromium was reduced significantly. The removal of Cr^{6+} was credited to the H_2S produced during growth, which acts as a reducing agent for Cr^{6+}. Smillie et al. (1981) reported a reduction of Cr^{6+} by bacterially produced H_2S in the marine environment. Fude and Shigui (1992) also confirmed that Cr^{6+} was reduced through a bacterially H_2S generation mechanism. Rahman et al. (2007) attributed the higher rate of Cr^{6+} reduction during the log phase of *Pseudomonas* sp.C-171 to faster growth and maximal H_2S production. By contrast, during the stationary phase, the accumulation of $Cr(OH)_3$ around the bacterial cells decreased the availability of H_2S for reaction with Cr^{6+}; this consequently decreased/stabilized the rate of Cr^{6+} reduction. Moreover, the degree of Cr^{6+} reduction was dependent upon the size of inoculum. An increase in inoculum concentration provided a higher number of bacterial cells, which in turn, increased the rate of H_2S production, thus facilitating Cr^{6+} reduction. The H_2S produced by bacteria reacts with chromium to form chromium sulfide, which is unstable in aqueous solution, and is rapidly precipitated as $Cr(OH)_3$.

Microbial mats are self-organized and laminated structures composed of heterotrophic and autotrophic organisms, and play a role in the bioaccumulation of toxic metals. Such mats, composed of cyanobacteria, purple bacteria (anoxygenic photosynthetic bacteria), and numerous heterotrophs, were capable of bioaccumulating approximately 100% of Cr^{6+} (Bender et al. 1995). Postgate (1984) studied the coupled oxidation of an energy source to sulfate reduction during growth in which sulfate was reduced (Postgate 1984) by SRB to sulfide (Lovely and Coates 1997; White and Gadd 1997, 1998). The cells of *Desulfovibrio desulfuricans* removed 86% of Cr^{6+} in 24 h, when the initial concentration was 500 μM (Turick et al. 1998). In contrast, 83% was of the initial 960 μM of Cr^{6+} removed in 5 days by using a mixed consortium SRB-III (Fude et al. 1994). Another SRB, in biofilm growth form, removed 88% Cr^{6+} in 48 h (Smith and Gadd 2000). Biofilm formation and growth confers more tolerance under environmental stress (e.g., desiccation and elevated levels of organic and inorganic pollutants), as compared to suspended growth forms (Costerton et al. 1994). However, less than 10% of precipitated chromium was retained by suspended cells, whereas, SRB-III retained most of the chromium as a precipitate around the cells. Fude et al. (1994) suggested that chromate was indirectly reduced by H_2S, and the precipitate was lodged in the cell wall. Absence of such a mechanism may have resulted in insignificant accumulation of chromium by the SRB (Smith and Gadd 2000).

7.3 Bioreduction

Microorganisms are capable of altering the oxidation/reduction state of toxic metals, organometal(oids), and radionuclide contaminants through direct or indirect biological and chemical process(es) (Gadd 1990; Gadd and White 1993; Lovely 1995). The mechanisms by which microorganisms reduce Cr^{6+} are variable, and are species specific. First, the Cr^{6+} reduction may be a part of the detoxification mechanism that occurs intracellularly. Second, some species use Cr^{6+} as an ultimate electron acceptor in the respiratory chain (Gvozdyak et al. 1986; Bopp and Ehrlich 1988; Wang et al. 1990), while in some other strains certain soluble enzymes are responsible for the reduction of Cr^{6+} to Cr^{3+} (Shen and Wang 1993; Wang and Xiao 1995; Philip et al. 1998; Park et al. 2000; Ackerley et al. 2004). Third, the reduction is an extracellular reaction, which occurs with excreted waste products, such as H_2S (Palmer and Puls 1994).

Biotransformation of Cr^{6+} to Cr^{3+} not only reduces the toxicity of chromium on living organisms, but also helps to precipitate chromium at a neutral pH for further physical removal.

Chromate (CrO_4^{2-}) is a strong oxidizing agent that is reduced in the presence of electron donors intracellularly to Cr^{5+}. Cr^{5+} then reacts with nucleic acids and other cell components to produce mutagenic/carcinogenic effects on biological systems (Clark 1994; McLean and Beveridge 2001). Although reduction to Cr^{5+} is responsible for chromate toxicity, its further reduction leads to the formation of a stable, less soluble, and less toxic trivalent chromium (Cr^{3+}). Therefore, reduction of Cr^{6+} to Cr^{3+} is a potentially beneficial process in Cr^{6+}-affected environments (Michel et al. 2001). Reduction of Cr^{6+} results in precipitation of chromium as Cr^{3+}, and thus offers to be a promising bioremediation strategy (Turick et al. 1998; Camargo et al. 2005).

Reduction of Cr^{6+} to Cr^{3+} has been demonstrated to occur in various bacterial species, including the following: *Bacillus* sp. (Campos et al. 1995; Camargo et al. 2004; Elangovan et al. 2006; Liu et al. 2006), *Pseudomonas* sp. (Bopp and Ehrlich 1988; Ishibashi et al. 1990; Suzuki et al. 1992; Salunkhe et al. 1998; Park et al. 2000; McLean and Beveridge 2001; Ganguli and Tripathi 2002; Farag and Zaki 2010), *E. coli* (Bae et al. 2005), *Desulfovibrio* sp. (Mabbett and Macaskie 2001), *Microbacterium* sp. (Pattanpipitpaisal et al. 2001), *Shewanella* sp. (Myers et al. 2000; Viamajala et al. 2002), *Arthrobacter* sp. (Megharaj et al. 2003; Asatiani et al. 2004), and *Staphylococcus aureus* and *Pediococcus pentosaceus* (Ilias et al. 2011). Bacteria that grow in a Cr^{6+}-containing natural environment develop chromium resistance and means to reduce accumulated Cr^{6+}; such behavior provides a clue that can be helpful in finding, isolating, and screening for microbes that have high reducing ability, which exist in Cr^{6+}-contaminated environments (Liu et al. 2006). However, Cr^{6+} reduction and resistance may not be closely correlated (Ohtake et al. 1987). There are many examples wherein chromate resistance results from the presence of efflux mechanisms which allow resistant strains to extrude CrO_4^{2-} ions (Bopp and Ehrlich 1988; Nies and Silver 1989; Ohtake et al. 1990b).

The transformation of Cr^{6+} to Cr^{3+} has been studied in *E. coli* (Shen and Wang 1994a; Ackerley et al. 2004), *Agrobacterium radiobacter* (Lovera et al. 1993), *P. fluorescens* LB3000, *Bacillus* sp., *Enterobacter cloacae* HO1, *E. aerogenes* (Thacker et al. 2006), *Shewanella* spp. (Viamajala et al. 2003), and *P. aeruginosa* (Xu and Liu 2005). A summary of the studies performed on the microbial reduction of hexavalent chromium under various experimental conditions is presented in Table 2. In these studies, glucose or organic acids were used as carbon and energy sources. Shakoori et al. (2000) studied the ability of a Gram-positive bacterium (probably *Bacillus* sp.) to reduce Cr^{6+} in a medium containing 20 ppm dichromate, and the authors observed >87% reduction within 72 h. Faisal et al. (2005) used two chromium-resistant bacterial strains, *B. cereus* S-6 and *Ochrobactrum intermedium* CrT-1 and two cyanobacterial strains, *Oscillatoria* sp. and *Synechocystis* sp. to reduce Cr^{6+} to Cr^{3+}. At initial chromate concentrations of 300 and 600 μg $K_2Cr_2O_7\,mL^{-1}$, and an inoculum size of 9.6×10^7 cells mL^{-1}, *B. cereus* completely reduced Cr^{6+}, while *O. intermedium* effected a reduction of 98% and 70%, respectively, after 96 h. At 100 μg $K_2Cr_2O_4\,mL^{-1}$, *Synechocystis* sp. and *Oscillatoria* sp., respectively reduced 62.1% and 39.9% of Cr^{6+}, at 30°C and pH 8.0.

When cell density is very high, a significant Cr^{6+} reduction is generally achieved. The rate of Cr^{6+} reduction increased with increasing cell density of *E. coli* ATCC33456 (Shen and Wang 1994a), *P. fluorescens* LB300, *Bacillus* sp. (Wang and Xiao 1995), *A. radiobacter* (Lovera et al. 1993), and *Enterobacter cloacae* (Wang et al. 1991). However, the specific rate of Cr^{6+} reduction by *E. coli* was higher at relatively lower cell densities, with the maximum reduction rate of 86 mg $Cr^{6+}\,h^{-1}\,mg^{-1}$ dry wt. at a cell density of 3×10^8 cells mL^{-1} (Shen and Wang 1994a). Cr^{6+} reduction occurs equally in cells at rest or in the growth phase. Resting cells of *P. fluorescens* (Bopp and Ehrlich 1988), *E. coli* (Shen and Wang 1994a), and *A. radiobacter* (Lovera et al. 1993) reduced Cr^{6+} at the same rate as occurred in growth media.

Ganguli and Tripathi (1999) evaluated the ability of a *P. aeruginosa* strain, isolated from tannery effluent, to survive and reduce chromate in effluents from a tannery and from an electroplating unit. The isolate survived in the native tannery effluent, but the count sharply declined in both native and diluted (200×) electroplating effluents. Supplementation with a source of carbon, nitrogen, and phosphorus enhanced bacterial cell number in both tannery and diluted electroplating effluents. Increased cell numbers directly correlated with increased chromate reduction in both tannery and diluted electroplating effluents. Camargo et al. (2003) isolated and screened 43 chromium-tolerant isolates. From these, five *Bacillus* sp. strains were found to be efficient chromium reducers. The growth rate of the isolates and their Cr^{6+}-reduction capacity was dependent on pH, temperature, and the chromium concentration. Maximum Cr^{6+} reduction by these strains was observed to occur at pH that ranged from 7.0 to 9.0, and at a temperature of 30°C. *Bacillus* sp. ES 29 aerobically reduced 90% Cr^{6+} within 6 h. Using the optimal pH for growth corresponded with the highest rate of Cr^{6+} reduction in most of these isolates. This was not surprising, because chromate (CrO_4^{2-}) is the dominant Cr^{6+} species in aquatic environments that have pH between 6.5 and 9.0 (McLean and Beveridge 2001). Since Cr^{6+} reduction is enzyme-mediated, changes in pH and temperature

Table 2 Microbial hexavalent chromium reduction under various optimized experimental conditions

Microorganisms	Source of organism	Experimental conditions						Chromium removal (%)	Reference
		System (medium/ effluent)	Initial Cr^{6+} concentration (mg $L^{-1)}$	Initial inoculum size (%v/v)/ cfu mL^{-1}	pH	Tempe- rature (°C)	Time (h)		
Pseudomonas aeruginosa A2Chr	Effluent of a leather tanning unit	Shake flask (tannery effluent)	40	8×10^8	–	37	35	60	Ganguli and Tripathi (1999)
CMB-Cr1 (ATCC 700729)	Wastewater from ponds receiving effluent from tanneries	Shake flask (LB broth)	20	10	9.0	37	72	87	Shakoori et al. (2000)
Bacillus sp. ES29	Potassium dichromate-contaminated soil sample from a land farming site	Shake flask (LB broth)	0.5 (500 μg L^{-1})	–	9.0	30	24	100	Camargo et al. (2003)
Arthrobacter sp.	Long-term tannery waste disposal site	Shake flask (MSM)	50	–	7.1	21	46	~60	Megharaj et al. (2003)
Strain CrT-11	Tannery effluent	Shake flask (Deleo and Ehrlich medium)	100	2.4×10^7	7.0	37	72	74.12	Faisal and Hasnain (2004)
				9.6×10^7	7.0	37	72	86.25	
Strain CrT-12			100	2.4×10^7	7.0	37	72	100	
				9.6×10^7	7.0	37	72	100	
Enterobacter cloacae	Marine sediment sample	Seawater medium	25	10^8	–	Room	80	63	Iyer et al. (2004)
			50	10^8	–	temperature	80	68	
			100	10^8	–		80	75	
Bacillus sp. XW-2	Chromium landfill	Shake flask (nutrient broth)	40	–	9.0	37	96	100	Liu et al. (2006)
Bacillus sp. XW-4			40	–	9.0	37	96	100	

Bacillus cereus S6	Chromium contaminated soil sample	Shake flask (Deleo and Ehrlich medium)	100	2.4×10^{7}	7.0	37	72	100	Faisal and Hasnain (2006)
				9.6×10^{7}			48	100	
Micrococcus sp.	Soil samples collected from 30-year-old small scale electroplating industry	Shake flask	100	–	7.0	35	24	90	Congeevaram et al. (2007)
Aspergillus sp.			100	–	5.0	35	24	92	
Bacillus sp.ev3	Wastewater	Shake flask (LB broth)	100	–	7.0	37	96	91	Rehman et al. (2007)
Bacillus cereus	Long term chromium polluted site	Shake flask (synthetic medium)	250 μM	2	7.0	30	30	100	Desai et al. (2008a)
Bacillus fusiformis			250 μM	2	7.0	30	30	100	
Bacillus sphaericus			250 μM	2	7.0	30	30	100	
Bacillus cereus			500 and 750 μM	2	7.0	30	30	95	
Bacillus fusiformis			500 and 750 μM	2	7.0	30	30	95	
Bacillus sphaericus			500 and 750 μM	2	7.0	30	30	95	
Bacillus cereus			1,000 μM	2	7.0	30	30	91	
Bacillus fusiformis			1,000 μM	2	7.0	30	30	95	
Bacillus sphaericus			1,000 μM	2	7.0	30	30	87	
Pseudomonas sp. G1DM21	Long term chromium polluted landfill site	Shake flask (LB broth)	250 μM	–	7.0	37	48	>99	Desai et al. (2008b)
			500 μM	–	7.0	37	48	>99	
			750 μM	–	7.0	37	48	>93	
			1,000 μM	–	7.0	37	48	>93	
Bacillus firmus KUCr1	Cr-containing soil sample	Shake flask (PYG medium)	2 mM	–	7.2	37	96	100	Sau et al. (2008)
Bacillus brevis	Tannery effluent	Shake flask (nutrient broth)	180	3.7×10^{6}		35	28	75.8	Verma et al. (2009)

(continued)

Table 2 (continued)

Microorganisms	Source of organism	Experimental conditions						Chromium removal (%)	Reference
		System (medium/ effluent)	Initial Cr^{6+} concentration (mg $L^{-1)}$	Initial inoculum size (%v/v)/ cfu mL^{-1}	pH	Tempe- rature (°C)	Time (h)		
Pseudomonas aeruginosa CCTCC AB 91095	Culture collection (CCTCC)	Shake flask (nutrient medium)	40	1	7.0	37	72	55	Xu et al. (2009)
Azotobacter chroococcum	Sewage effluent and sewage irrigated soils	Skake flask (nutrient broth)	100	–	–	35	72	69.20	Parameswari et al. (2009)
Bacillus sp.			100	–	–	35	72	78.56	
Pseudomonas fluorescens			100	–	–	35	72	80.21	
Bacillus subtilis	–	Shake flask (tannery effluent)	570	1	7.0	37	192	99.6	Benazir et al. (2010)
Pseudomonas aeruginosa			570	1	7.0	37	192	99.6	
Saccharomyces cerevisiae			570	1	7.0	37	192	95.6	
Pseudomonas aeruginosa + *Bacillus subtilis*			570	1	7.0	37	192	99.6	
Saccharomyces cerevisiae + *Bacillus subtilis*			570	1	7.0	37	192	97.2	
Saccharomyces cerevisiae + *Pseudomonas aeruginosa*			570	1	7.0	37	192	99.3	

Bacillus sp. (CSB-4)	Chromite mine soil	Shake flask (LB broth)	100	–	~7	35	144	~ 90.0	Dhal et al. (2010)
Brevibacterium casei	Deposit of chromite ore	Shake flask (MSM)	50	–	7.0	30	12	98	Das and Mishra (2010)
Acinetobacter sp.	Tannery effluent	Shake flask (LB broth)	200	2	7.0	30	72	53.5	Farag and Zaki (2010)
Pseudomonas sp.			200	2	7.0	30	72	62	
Corynebacterium paurometabolum	Mine seepage water	Shake flask (KSC medium)	2 mM	10^6	7.0	35	–	>90	Dey and Paul (2010)
Bacillus sp. Strain FM1	Tannery effluent	Shake flask (LB broth)	100	Log phase culture	8.0	37	48	100	Masood and Malik (2011)

will affect both the ionization and conformation of proteins, thereby affecting enzyme activity (Farrell and Ranallo 2000).

Megharaj et al. (2003) isolated *Bacillus* and *Arthrobacter* spp. from long-term tannery-waste contaminated soil, and evaluated the degree of tolerance they had to Cr^{6+} and the capacity they retained to reduce chromium. The time required to reduce Cr^{6+} increased as Cr^{6+} concentration increased. The Cr^{6+} reducing ability of the bacteria was growth-dependent, and *Arthrobacter* sp. was a more efficient reducer than *Bacillus* sp. Assays with permeabilized (treated with toluene or Triton X-100) cells and crude extracts demonstrated that Cr^{6+} reduction was mainly associated with the soluble fraction of the cells. Faisal and Hasnain (2004) isolated highly Cr^{6+} tolerant (25 mg mL^{-1} in nutrient broth and 40 mg mL^{-1} in nutrient agar) bacterial strains (CrT-11, CrT-12, *Bravibacterium* sp. CrT-13 and CrT-14) from tannery effluent. These strains were inoculated at two cell densities (viz., 2.4×10^7 and 9.6×10^7 colony forming units (cfu) mL^{-1}). All of the above strains aerobically reduced 74.12–100% of Cr^{6+} to Cr^{3+} (from an initial 100 μg Cr^{6+} mL^{-1} concentration) within 72 h. The Cr^{6+} reduction potential of these strains was also tested in industrial effluent from a metal finishing process that contained an initial chromate concentration of 150 μg mL^{-1}; other metal pollutants were also present in the effluent. The following strains that were tested included CrT-11, CrT-12, *Bravibacterium* sp. CrT-13, and CrT-14. These, respectively, reduced approximately 71.10%, 93.12%, 68.2%, and 79.0 % of Cr^{6+} within 40 h.

Campos et al. (2005) studied the aerobic chromate reduction potential of *Serratia marcescens* that was isolated from tannery effluent. These authors noted an 86% reduction (from 0.5 mM Cr^{6+} initially to Cr^{3+}) after 48 h, and at a rate of 1.51 mg L^{-1} h^{-1}. Faisal and Hasnain (2006) reported that *B. cereus* S-6 strain reduced Cr^{6+} to Cr^{3+}, and the concentration of Cr^{6+} at all the initial three levels (0.1, 0.5, 1.0 mg mL^{-1}) decreased as incubation time increased. At a low initial concentration of 0.1 mg Cr^{6+} mL^{-1}, the strain was able to reduce 100% of the Cr^{6+} within 48 h. However, the rate of Cr^{6+} reduction that occurred in the industrial electroplating effluent was slightly less, which was supposedly due to the presence of other pollutants and a high concentration of chromate ions. With an initial chromate concentration of 0.15 mg Cr^{6+} mL^{-1} electroplating effluent, the strain reduced 94% of Cr^{6+}, whereas at initial concentration of 0.3 mg Cr^{6+} mL^{-1}, the strain reduced nearly 82% of Cr^{6+} within 40 h.

Quazilbash et al. (2006) isolated six *Bacillus* strains (QIP 1–6) that exhibited maximal growth rates when their media contained 400 ppm Cr^{6+}. Strains QIP1 and QIP5 exhibited the maximum resistance to Cr^{6+} at 900 ppm; at this loading, 46.81% and 42.50% of Cr^{6+} was, respectively, removed under optimal conditions. Liu et al. (2006) studied Cr^{6+} reduction by a *Bacillus* sp. XW-4 isolate, and reported that maximum reduction occurred at an optimum pH of 9.0 and a temperature of 37°C. Further, within the tested range (10–80 mg Cr^{6+} L^{-1}), increased reduction occurred at higher initial concentrations, and at the same incubation time of 72 h. Aravindhan et al. (2007) reported an aerobic reduction of Cr^{6+} to Cr^{3+} by employing *Pseudomonas* cultures that had mixed chromium resistance (up to 300 mg L^{-1}), and were isolated from marshy land. A direct correlation was observed between the increasing concentration of Cr^{6+} and time required for complete reduction (which was 6 h at

initial 15 mg Cr^{6+} L^{-1}, 24 h for 25–50 mg L^{-1}, and >48 h for Cr^{6+} at above 100 mg L^{-1}). NADH enhanced the rate of Cr^{6+} reduction, while SO_4^{2-} and poly-phenols inhibited the metabolic activity related to Cr^{6+} reduction (Aravindhan et al. 2007).

Desai et al. (2008a) isolated three Cr^{6+} reducing bacterial strains, *B. cereus*, *B. fusiformis*, and *B. sphaericus,* from a chromium polluted landfill. These strains reduced >85% of an initial Cr^{6+} concentration of 1,000 μM Cr^{6+} within 30 h. The Cr^{6+} reductase activity of these strains existed mainly in the soluble fraction of cells and was constitutively expressed. Supplementation of the cell-free extracts (from all the three isolates), in which they were tested with the addition of 1 mM of NADH enhanced the Cr^{6+} reductase activity. The reductase activity was stable in the presence of different metal ions tested except for two: Hg^{2+} and Ag^{+}. The authors (Desai et al. 2008b) thereafter isolated *Pseudomonas* sp. G1DM21, and reported that the culture reduced Cr^{6+} by 99.7% (from initial 500 μM) and 93.06% (from initial 1,000 μM) within 48 h. The culture consecutively reduced four inputs of 100 μM Cr^{6+} each, within 6 h. Further, the permeabilized cells of the bacterium reduced 92% of 100 μM Cr^{6+} within 6 h, whereas the cell-free extract reduced 90% of 100 μM Cr^{6+} within 2 h. The Cr^{6+} reductase activity increased in the presence of Cu^{2+}, Mg^{2+}, Na^{+} and electron donors like citrate, succinate, acetate, whereas there was significant inhibition in the presence of Hg^{2+}, Ag^{+}, Cd^{2+} and disulfide reducers like 2-mercaptoethanol. Rehman et al. (2008) reported that a *Bacillus* sp. ev3 isolate successfully reduced 91% of an initial 100 μg mL^{-1} Cr^{6+} at a pH of 7.0 and a temperature of 37°C, within 96 h. Similarly, 84% of Cr^{6+} from this industrial effluent was reduced at 144th hour. The cell-free extract of this isolate exhibited a reduction of 70%, 45.6%, and 27.4% at initial concentrations of 10, 50, and 100 μg Cr^{6+} mL^{-1}, respectively.

Xu et al. (2009) reported that the Cr^{6+} reduction effected by *P. aeruginosa* was correlated with bacterial growth, wherein the Cr^{6+} concentration gradually decreased as bacterial growth increased. Furthermore, a significant increase in bacterial growth was noted as Cr^{6+} was more efficiently reduced, indicating that Cr^{6+} reduction and bacterial growth affect each other. Thus, the growth of cells stimulated Cr^{6+} reduction, and in-turn, the efficient Cr^{6+} reduction promoted bacterial growth. Polti et al. (2009) determined the extent of Cr^{6+} reduction by *Streptomyces* sp. MC1 in minimal liquid media, soil extracts, and soil samples. In the minimal liquid medium, 100% and 75% Cr^{6+} reduction was obtained (at initial 5 and 50 mg Cr^{6+} L^{-1}, respectively) within 48 h incubation. The isolate also reduced 30% Cr^{6+} from the initial 10 mg Cr^{6+} L^{-1} level in soil extracts within 96 h, and 94% of Cr^{6+} from the initial 50 mg Cr^{6+} kg^{-1} levels in soil samples on the 7th day. The reduction took place during the exponential growth phase, and occurred at a pH of 7 and at a temperature of 30°C. The authors subsequently characterized a constitutive chromate reductase from the MC1 strain (which was mainly associated with the biomass); NAD(P)H was required as an electron donor for enzymatic activity (Polti et al. 2010).

Das and Mishra (2010) observed a bacterial growth-dependent reduction of 99% for Cr^{6+} by *Bravibacterium casei* (from initial 50 mg Cr^{6+} L^{-1} medium) within 12 h; optima for pH was 7.0 and for temperature 30°C. He et al. (2010) isolated *B. cereus* SJ1 from the chromium-contaminated wastewater of a metal electroplating factory, and this organism exhibited complete reduction of Cr^{6+} at an initial

1 mM level, within 57 h. Dhal et al. (2010) isolated 13 Cr^{6+}-resistant bacterial strains from an Indian chromite mine soil; *Bacillus* sp. was the most metal tolerant (2,000 mg Cr^{6+} L^{-1}) isolate (CSB-4). This strain reduced 90% of the Cr^{6+} present (from the initial 100 mg Cr^{6+} L^{-1} level) and approximately 100% of an initial ≤50 mg L^{-1} level, in 144 h. Optima for this incubation were as follows: a pH of 7.0, temperature of 35°C, and an agitation speed of 100 rpm. Further, the rate of Cr^{6+} reduction decreased with time (especially with higher initial Cr^{6+} concentration), presumably from the Cr^{6+} toxicity on bacterial cells. Dey and Paul (2010) isolated and selected ten species of *Pseudomonas*, *Acinetobacter*, *Alcaligenes*, *Cupriavidus*, and *Corynebacterium* on the basis of chromate reducing efficiencies. The *Corynebacterium paurometabolum* SKPD 1204 (MTCC 8730) exhibited the maximum Cr^{6+} reducing capacity of 63.7% in Vogel Bonner (VB) broth and 92% in KSC media. This organism was also capable of reducing ~95% of the Cr^{6+} present in mine seepage supplemented with a 2% VB concentrate.

A variety of organic compounds are known to donate electrons for the microbial reduction of chromium (Ohtake et al. 1990a; Wang and Xiao 1995; Bhide et al. 1996; Philip et al. 1998; Ganguli and Tripathi 1999; Liu et al. 2004). The majority of these electron donors are small molecular weight natural aliphatic compounds such as carbohydrates, amino acids, and fatty acids (Shen and Wang 1995). Liu et al. (2006) studied the effect of glucose supplementation on Cr^{6+} reduction by *Bacillus* sp. XW-4, and reported a significant decrease in the level of Cr^{6+} (from the initial 40 to 4.24 mg L^{-1}), during a 72-h incubation. The presence of glucose dramatically decreased the final pH of the test system to 5–6 after 72-h incubation. The effect on final pH did not occur without glucose. This indicates the profound role that glucose plays on bacterial metabolism and on Cr^{6+} reduction.

Sau et al. (2008) reported that a *B. firmus* isolate exhibited better growth in a glucose-supplemented medium, which in turn, enhanced Cr^{6+} reduction. The bacterium grew significantly better when additional nutrients (N and P) were used to supplement tannery wastewater, consequently doubling Cr^{6+} reduction, from 32.5% under nutrient-deficient conditions to 64.4% under nutrient-supportive conditions. Masood and Malik (2011) studied the effect of glucose on Cr^{6+} reduction by *Bacillus* sp. FM1. They reported a decrease in the concentration in glucose-supplemented media of 28.0 ± 1.5 mg Cr^{6+} L^{-1} (from the initial 100 mg Cr^{6+} L^{-1}). In contrast, the decrease in the glucose-free medium was 37.2 ± 1.8 mg Cr^{6+} L^{-1}. Pattanapipitpaisal et al. (2001) isolated an anaerobically Cr^{6+} reducing *Microbacterium* sp. MP 30, which completely reduced a titer of 100 μM Cr^{6+} within 72 h, at the expense of acetate as an electron donor.

Biological reduction of Cr^{6+} may occur either aerobically (Bopp and Ehrlich 1988; Ishibashi et al. 1990; Cooke et al. 1995; Wang and Xiao 1995), anaerobically (Lebedeva and Lyalikova 1979; Wang et al. 1989) or both, i.e., facultatively (Lovera et al. 1993; Shen and Wang 1993; Jeyasingh and Philip 2005; Rama Krishna and Philip 2005; Chai et al. 2009; Elangovan and Philip 2009; Shukla et al. 2009). However, the anaerobic reduction of Cr^{6} is more common. Aerobic Cr^{6+} reduction remains generally associated with cytosolic soluble proteins that employ NADH as an electron donor (Campos et al. 1995; Cervantes et al. 2001). In the absence of added

electron donors, endogenous reserves are used for the reduction of Cr^{6+} (Horitsu et al. 1987; Ishibashi et al. 1990; Shen and Wang 1993; Wang and Xiao 1995). Aerobically, the dissolved oxygen itself causes an uncompetitive inhibition of Cr^{6+} reduction in many strains (Wang and Shen 1995). Since Cr^{6+} reduction consumes energy from the respiratory chains, it is affected by numerous environmental factors. Anaerobic chromate reduction occurs at the cytoplasmic membrane (Wang et al. 1991; McLean and Beveridge 2001), wherein cyt *c* in *E. cloacae* (Wang et al. 1989), cyt *b* and *d* in *E. coli* (Shen and Wang 1994a), and cyt c_3 in *Desulfovibrio vulgaris* (Lovely and Phillips 1994) serve as terminal electron acceptors.

Lovely and Phillips (1994) observed the reduction of Cr^{6+} in the anaerobic autotroph *Desulfovibrio vulgaris* ATCC 29579, when molecular H_2 was used as an electron donor. However, in this same study, a pure culture of *E. coli* that did not utilize molecular hydrogen also reduced Cr^{6+}, suggesting that another reduction pathway for Cr^{6+} existed. The cells of *A. radiobacter* EPS-916 (Lovera et al. 1993), *E. coli* ATCC33456 (Shen and Wang 1993), species of *Aerococcus*, *Aeromonas* and *Micrococcus* (Srinath et al. 2001) were reported to reduce Cr^{6+} both aerobically as well as anaerobically; however, a better reduction occurred in the absence of oxygen. Srinath et al. (2001) isolated several facultative anaerobes that exhibited varying degrees of Cr^{6+} reduction capability, under both aerobic and anaerobic conditions at ambient temperature (24 ± 2°C). Eight isolates, viz., five strains of *Aerococcus* sp., two of *Micrococcus* sp., and a single strain of *Aeromonas* sp. were capable of anaerobically reducing 70% of the initial 20 mg Cr^{6+} L^{-1} within 3 days.

In *Enterobacter cloacae* HO1, the reduction of Cr^{6+} was slightly inhibited by sulfate (at 120 mg L^{-1}) and nitrate (at 150 mg L^{-1}), under anaerobic condition (Shen and Wang 1994a). The specific activity of chromate reductase from *Pseudomonas mendocina* MCM B-180 was decreased by nitrite, nitrate, and sulfate ions (Rajwade et al. 1999). In contrast, the nitrate, at levels as high as 2,240 mg L^{-1} had no effect on Cr^{6+} reduction by *E. coli* (Shen and Wang 1994a). Similarly, no inhibition of Cr^{6+} reduction was evident, even at 4,000 and 5,760 mg SO_4^{2-} L^{-1} levels for *E. coli* (Shen and Wang 1994a) and *E. cloacae* (Komori et al. 1989, 1990), respectively. The sulfate (at 1 mM) and nitrate (at 200 μM) had no effect on chromate reduction by whole cells or cell-free supernatant of *Pseudomonas putida* (Ishibashi et al. 1990). Philip et al. (1998) found that the presence of sulfate or nitrate, up to levels of 1,000 mg L^{-1}, did not affect Cr^{6+} reduction by *B. coagulans*. Wang and Xiao (1995) reported that the Cr^{6+} reduction activity of a *Bacillus* cell-extract was unaffected by the addition of 1,000 mg SO_4^{2-} or NO_3^- L^{-1}. Furthermore, neither 1,200 mg SO_4^{2-} nor 1,400 mg NO_3^- L^{-1}, influenced Cr^{6+} reduction, even by whole cells of *Bacillus* sp. Liu et al. (2006) and Xu et al. (2009) could not find any effect of NO_3^- and SO_4^{2-} (at 40 and 80 mg L^{-1}) on Cr^{6+} reduction by *Bacillus* sp. XW4 and *P. aeruginosa*.

In general, the inhibition of Cr^{6+} reduction by sulfate or nitrate has not been reported for aerobic cultures, because they do not act as electron acceptors. Hence, they would not compete with Cr^{6+} for accepting electrons under aerobic conditions (Wang and Xiao 1995; Garbisu et al. 1998; Liu et al. 2006). In contrast, nitrate and sulfate generally produce an effect on the reduction of Cr^{6+} under anaerobic conditions (Liu et al. 2006), because they may compete with chromate as anaerobic

electron acceptors (Garbisu et al. 1998). Additionally, inhibition by sulfate may result from competition in the sulfate transport pathway, owing to the chemical similarity between CrO_4^{2-} and SO_4^{2-} (Chen and Hao 1996).

The rate at which Cr^{6+} is reduced decreases as pH increases and increases with increasing initial Cr^{6+} concentration. At a neutral pH, many weeks may be required for Cr^{6+} to be completely reduced (Palmer and Puls 1994). The influence of Cr^{6+} concentration on its own reduction appears to indicate two opposing trends. First, the time required for complete reduction increases progressively with an increase in initial Cr^{6+} concentration in the following species: *E. cloacae* (Wang et al. 1989), *E. coli* (Shen and Wang 1994a) and *Bacillus* sp. (Wang and Xiao 1995; Sau et al. 2008). Second, a slower initial rate of Cr^{6+} reduction occurs at higher initial Cr^{6+} concentrations (Komori et al. 1989, 1990).

The effect on Cr^{6+} reduction of altering the initial Cr^{6+} concentrations in five *Bacillus* species isolates indicated that the reduction was possibly mediated via enzymatic activity; the fit of the reduction data and hyperbolic shape of the curve revealed a clear dependence of reduction on Cr^{6+} concentration (Camargo et al. 2003). Liu et al. (2006) observed that more Cr^{6+} was reduced by *Bacillus* sp. XW-4 at higher initial Cr^{6+} concentration, even for the same incubation times. Sau et al. (2008) reported that Cr^{6+} was almost completely reduced at all tested concentrations (0.5–2 mM Cr^{6+}) within 120 h by *B. firmus* KUCr1. At lower Cr^{6+} concentrations (0.5 and 1.0 mM), the reduction rate increased sharply up to 24 h, and Cr^{6+} was completely reduced within 48 h. However, at higher Cr^{6+} concentrations of 1.5 and 2.0 mM, although the Cr^{6+} reduction rate was slow during the first 24 h, complete reduction was effected at 72 and 120 h, at 1.5 and 2.0 mM, respectively. Masood and Malik 2011 reported the complete reduction of Cr^{6+} (from initial levels of 50 and 100 mg Cr^{6+} L^{-1}) by *Bacillus* sp. strain FM1 at 12 and 48 h, respectively. The longer period (48 h) taken for Cr $^{6+}$ reduction may be attributed to the toxic effect of the higher Cr^{6+} concentration on growth of the strain FM1.

The susceptibility of Cr^{6+} reducing organisms to the presence of one or more other heavy metals has been reported by several researchers. Such heavy metals are concomitantly present in wastewaters of various industrial processes. Metals such as Cu^{2+}, Cd^{2+}, Ni^{2+}, Zn^{2+} (Ohtake et al. 1990b; Chen and Hao 1996; Garbisu et al. 1997; Faisal and Hasnain 2004, 2006; Masood and Malik 2011), Hg^{2+}, Ag^{2+} (Ishibashi et al. 1990), Mo^{6+} and Fe^{3+} (Lovera et al. 1993) exhibited varied patterns of inhibition, depending on the species and strain of bacteria tested. Appanna et al. (1996) reported that Fe^{3+} supplementation was essentially required for detoxification of Cr^{6+} by *P. fluorescens* ATCC 13525. Bae et al. (2000) reported that 1 mM Zn^{2+} L^{-1} caused a 32% decrease in the reduction of Cr^{6+} by *E. coli*. Desjardin et al. (2003) observed that Ni and Cd induced Cr^{6+} reduction by *Streptomyces thermocarboxydus* during a 72-h incubation.

Faisal and Hasnain (2004) reported that Zn^{2+} at 200 μg mL^{-1} slightly stimulated Cr^{6+} reduction by CrT-11, CrT-12, *Bravibacterium* sp., CrT-13, and CrT-14 strains. In contrast, Sultan and Hasnain (2007) reported a clearer inhibitory effect of Zn^{2+} on Cr^{6+} reduction by *O. intermedium*. Sau et al. (2008) reported varied effects of different heavy metals (at 0.1 mM level) on Cr^{6+} reduction by a *B. firmus* KUCr1 isolate.

Cd and Zn significantly inhibited, whereas arsenic, cobalt, and nickel did not affect, Cr^{6+} reduction, although all treatment regimes yielded similar cell mass. Xu et al. (2009) also noted that, in the presence of Zn^{2+} (at 100 and 25 mg L^{-1}), Cr^{6+} decreased from 40 mg L^{-1} to only 26–27 mg L^{-1}, whereas Cr^{6+} decreased to 1 mg L^{-1} in the absence of Zn^{2+} within 96 h by *P. aeruginosa*. Cr^{6+} reduction by *Bacillus* sp. FM1 was significantly inhibited (35%, 49.37%, and 55.65%,) by Cd^{2+}, Zn^{2+}, and Cu^{2+} , respectively, whereas, Co^{2+} and Ni^{2+} exhibited a negligible effect (Masood and Malik 2011).

The Cr^{6+} reduction by cell-free extract of *Bacillus* sp. ES 29, and whole cells of *O. intermedium,* was stimulated by Cu^{2+} (Camargo et al. 2003; Sultan and Hasnain 2007). The presence of Cu^{2+} stimulated Cr^{6+} reductase activity of *Pseudomonas* sp.G1DM21 by 33% (Desai et al. 2008b). Sau et al. (2010) detected constitutive type chromium reductase activity both in membrane and soluble cell fractions of *B. firmus* KUCr1. Further, metal ions of Cu^{2+}, Co^{2+}, Ni^{2+}, and As^{3+} stimulated, while, Ag^{+}, Hg^{2+}, Zn^{2+}, Mn^{+}, Cd^{2+}, and Pb^{2+} inhibited Cr^{6+} reductase activity. Xu et al. (2009) reported a substantial stimulatory effect of Cu^{2+} on Cr^{6+} removal (from initial 40 to 1–2 mg L^{-1}) by *P. aeruginosa* within 48 h.

The mechanism by which Cu^{2+} stimulates Cr^{6+} reduction is not clear. However, Cu^{2+} is a transition metal, and is a prosthetic group for many reductase enzymes. The major function of Cu^{2+} is related to either the protection of electron-transport or as a single-electron redox center. In some cases, Cu^{2+} also serves as a shuttle for electrons between protein subunits (Camargo et al. 2003; Xu et al. 2009). In contrast, Ohtake et al. (1990a) found that 0.5 mM Cu^{2+} L^{-1} inhibited 32% of Cr^{6+} reduction by a membrane-bound chromate reductase under anaerobic conditions. Shen and Wang (1994b) also reported that the presence of 100 mg Cu^{2+} L^{-1} caused 16–33% decrease in Cr^{6+} reduction by *E. coli*, but not at lower concentrations. Faisal and Hasnain (2006) observed a slight inhibitory effect from Cu^{2+} and Co^{2+}, while Zn^{2+} and Ni^{2+} at 200 μg mL^{-1} stimulated the reduction of Cr^{6+} by *B. cereus* S-6. However, these metals at low concentrations had no effect on Cr^{6+} reduction capability.

8 Bioremediation by Immobilized Biomass and Elution of Chromium

The cell biomass used for bioremediation is normally loaded with metals. If successful recovery of such metals from biomass is not ensured, the bioremoval process would be nothing more than the transfer of heavy metals from one component (the environment) to another (cell biomass). Moreover, regenerated biomass can be more economic if it is reused for subsequent round(s) of biosorption. However, if biomass is to be reused there must be an economically viable recovery process for it. To achieve the stipulated goal of bioremediation and recovery of metal, one option is to immobilize the biomass. Biomass is immobilized by attaching or entrapment of microbial cells onto an inert and insoluble support material (matrix). By using immobilization, several processing steps become easier. For example, it is easier to

separate biosorbent from effluent, to repeat biosorption cycles, and to elute (desorb) metals from the biomass (Pethkar and Paknikar 1998; Puranik and Paknikar 1999).

When free cells are employed for bioremediation, they often suffer from excessive Cr^{6+} toxicity and cell damage. Furthermore, when free-cell biomass is used (as a biosorbent) in commercial processes, there may be problems associated with the physical characteristics of the cells, such as small size, low density, poor mechanical strength/rigidity, and solid–liquid separation. These problems can be resolved by immobilizing microbial cells on natural or synthetic polymer matrices. However, natural matrices are biodegradable and are prone to abrasion. Therefore, choosing the proper immobilization support is a key factor in building an optimal immobilized biocatalyst (Poopal and Laxman 2008). The use of expensive electron donors is not feasible in large-scale bioremediation projects. Hence, immobilization of whole cells that utilize endogenous electron donors is advantageous. By using whole-cell immobilization, improvements over free cells are achieved in increased stability, ease of regeneration, possibility of reuse, easier solid–liquid separations, and minimum clogging in continuous systems (Viraraghavan and Kapoor 1998; Zhao and Duncan 1998; Aksu et al. 1999; Valdman et al. 2001; Aksu and Gonen 2004; Arica and Bayramoglu 2005). The benefits of selecting the best cell immobilization option for bioremediation is so important, that it has attracted the attention of many researchers worldwide.

The ability to elute bound chromium from cell biomass is affected by its ionic state. When chromium is bound in the hexavalent state, it can be easily eluted with acidic solutions. These solutions reduce the metal to the trivalent state, which subsequently is released into the eluent fraction. However, 100% elution of Cr^{3+} was not achieved from *Sargassum* biomass (Kratochvil et al. 1998), probably due to the slow reduction of biomass-bound Cr^{6+} to Cr^{3+}. However, higher chromium recovery was achieved from biomass that was equilibrated to higher concentrations of Cr^{6+}.

Akthar and Mohan (1995) reported that alkali-treated biomass of *A. niger* remediated >95% of Cr^{6+} (from initial 420 mg L^{-1}) from electroplating effluent in five successive cycles. The biosorbed chromium was eluted with 0.1N HCl. However, the chromium elution efficiency decreased during subsequent cycles. Conversely, Wilhelmi and Duncan (1995) reported that a mild acidic solution (0.1 M HCl) did not elute biosorbed Cr^{3+} from *S. cerevisiae* biomass, although elution performance marginally increased (to 34%) upon increasing the concentration of the acid. Srinath et al. (2003a) found that 1.0 M sulfuric acid was the most efficient eluent for desorbing chromium from immobilized agarose-*B. coagulans* biomass. However, the biosorptive feature (metal loading capacity) of the regenerated immobilized biomass was reduced in the second cycle. This reduction in biosorption possibly resulted from the loss of matrix (agarose-*B. coagulans* biomass) weight during the elution process. It has been demonstrated in other studies that acids cause structural damage to the biomass, although the damage is far less than the damage caused by alkaline agents (Hu and Reeves 1997; Puranik and Paknikar 1997).

In Table 3, we summarize the extent to which Cr^{6+} has been bioremediated by immobilized microbial cells and various immobilization matrices. The cells of

Table 3 Bioremediation of hexavalent chromium by immobilized microbial biomass

	Experimental conditions							Chromium		
Microbial biomass	Immobilization matrix	System (medium/effluent)	Initial Cr^{6+} concentration (mg L^{-1})	pH	Temperature (°C)	Cycles	Time (h)	Per cent removal	Uptake rate (mg g^{-1} bios)	Reference
Bacillus coagulans	Agar	Shake flask [Cr^{6+} working solution]	100	2.5	28	9	2	–	33.43	Srinath et al. (2003a)
	Calcium alginate		100	2.5	28	9	2	–	35.43	
	Agarose		100	2.5	28	9	2	–	40.56	
	Polyacrylamide		100	2.5	28	9	2	–	40.58	
Pseudomonas sp.	Sodium alginate	Shake flask [Cr^{6+} working solution]	100	–	–	–	4	66.55	–	Murugesan and Maheshwari (2007)
Streptomyces griseus	PVA-alginate	Shake flask (Broth-II medium)	25	–	28	5	24	100	–	Poopal and Laxman (2008)
	Agarose		24.9	–	28	5	24	96.8	–	
	Agar		25.9	–	28	5	24	90.4	–	
	Polyacrylamide-alginate		24.8	–	28	5	24	80.3	–	
	PVA-borate		25.8	–	28	5	24	44.6	–	
	PVA-nitrate		25.5	–	28	5	24	28.6	–	
Pseudomonas strain S4	Ca-alginate	Shake flask (LB broth)	120	7.0	30	3	168	85.11	–	Farag and Zaki (2010)
Bacillus subtilis	Sodium alginate	Shake flask (tannery effluent)	570	7.0	Room temperature	–	192	99.6	–	Benazir et al. (2010)
Pseudomonas aeruginosa	Sodium alginate		570	7.0		–	192	99.3	–	
Saccharomyces cerevisiae	Sodium alginate		570	7.0		–	192	100	–	

Microbacterium liquefaciens, immobilized in polyvinyl alcohol, effected 90–95% Cr^{6+} removal (from initial 50 μM Cr^{6+}) within 20 days (at a steady-state) in a flow-through column bioreactor (Pattanapipitpaisal et al. 2001). Ganguli and Tripathi (2002) compared the chromate removal ability of batch culture (free cells) and agarose-alginate immobilized *P. aeruginosa* A2Chr biomass (from initial 10 to 100 mg Cr^{6+} L^{-1}) in a minimal medium and electroplating effluent. In batch culture study, the maximum Cr^{6+} removal of 9.4 mg L^{-1} occurred at the lowest initial Cr^{6+} concentration of 10 mg L^{-1} minimal medium, within 2 h. However, at higher initial Cr^{6+} concentrations of 50 and 100 mg L^{-1}, Cr^{6+} removal was only 7.6 and 2.1 mg L^{-1}, within 2 h. The removal of Cr^{6+} from electroplating effluent was approximately 25% lower than removal rates from the minimal medium.

Srinath et al. (2003a) compared the Cr^{6+} biosorption efficiencies of *B. coagulans* cells that were immobilized in different matrices such as agar-agar, calcium alginate, agarose, and polyacrylamide. The authors earlier (Srinath et al. 2002a) reported that the biosorption of Cr^{6+} was highly influenced by pH, effecting maximum sorption at pH 2.5. The stability of the matrix during biosorption was essential to prevent hampering Cr^{6+} sorption efficiency. At low pH, Cr^{6+} sorption was least affected when agarose and polyacrylamide immobilized biomass were used, and performed nearly equally as did the free cell biomass. These matrices were highly stable at a pH of 2.5. However, agar- and calcium alginate-immobilized biomass matrices exhibited low stability and decreased Cr^{6+} biosorption at acidic pH (Srinath et al. 2003a).

Similarly, Uchiyama et al. (1994) found that the integrity of agarose-immobilized biomass remained intact at acidic pH. However, owing to the cationic cross-link nature of alginate, biomass integrity was compromised by the presence of anions (such as chromate) under acidic conditions. Furthermore, agarose and polyacrylamide are known to provide good resistance to hydrostatic pressure and mechanical degradation (Gadd and White 1993). However, polyacrylamide is relatively less resistant to mechanical stress (Hu and Reeves 1997). Hence, agarose was a better option than polyacrylamide, and was, therefore, chosen as an immobilization matrix for further studies by Srinath et al. (2003a). After 24 h, equilibrium of *B. coagulans* biomass-agarose was established with Cr^{6+} solution; ~70% of the bound chromium was eluted using the eluent 0.1 M H_2SO_4. Conversely, the chromium elution efficiency for free cells of *B. coagulans* biomass was 86.11%. The interaction between the bound Cr^{6+} and the eluent was rather slow, as was the reduction of Cr^{6+} to Cr^{3+}; this slow reduction may have reduced the elution efficiency of the Cr^{6+} from the agarose immobilized biomass (Kratochvil et al. 1998), as compared to the free cells. Further, during the second biosorption–elution cycle, Cr^{6+} sorption was nearly comparable to that of the first exposure, in which ~80% Cr^{6+} was sorbed on the biomass-agarose; however, the elution efficiency of chromium in the second cycle was reduced to less than 50% that of the first cycle. The matrix started loosing its integrity during fifth cycle (Srinath et al. 2003a). The authors also evaluated the efficiency of agarose-immobilized *B. coagulans* biomass for removal of Cr^{6+} from the effluent of combined treatment plant (CETP), which resulted in complete remediation of the Cr^{6+} within five cycles.

Camargo et al. (2004) revealed that *Bacillus* sp. ES 29 cells immobilized on celite removed 98% of the influent Cr^{6+} (from the initial 2 to 8 mg Cr^{6+} L^{-1}), at flow rates of 3–6 mL h^{-1}. Humphries et al. (2005) reported that PVA-alginate-immobilized cells of *M. liquefaciens* required 4 days to reduce chromate from 100 to 2 μM concentrations, whereas agar-immobilized *Microbacterium* sp. NCIMB 13776 required 65 h to reduce 500–261 μM chromate. Murugesan and Maheswari (2007) reported a 66.55% chromium removal efficiency (at an initial 100 ppm Cr^{6+} concentration) for alginate-immobilized *Pseudomonas* sp. within 4 h. This efficiency was attributed to result from the better porosity of beads, which allowed free transport of metal ions through the matrix**.**

Poopal and Laxman (2008) reported that among the matrices tried for whole cell immobilization of *Streptomyces griseus*, PVA-alginate proved the most effective matrix, followed by agarose, agar, and polyacrylamide with Cr^{6+} reduction efficiencies of 100%, 96.8%, 90.4%, and 80.3%, respectively, in 24 h. But, the cells immobilized in PVA-nitrate and PVA-borate beads were unstable, and disintegrated within 24 h. Further, only ~ 28.6% and 44.6% Cr^{6+} was removed by the PVA-nitrate and PVA-borate immobilized cells, respectively, in 24 h. The Cr^{6+} removal efficiency did not increase further, even upon prolonged incubation. However, complete removal of Cr^{6+} was evident in 24 h incubations from both free- and PVA-alginate-immobilized cells, which were cycled for use in five batches, and completely reduced the initial 25 mg Cr^{6+} L^{-1} concentration each time. The reduction achieved during the sixth cycle was only 50% of what was removed in earlier cycles, which may have been due to loss in cell viability. Moreover, the initial 25 mg Cr^{6+} L^{-1} level was fully reduced by immobilized cells taken from a simulated effluent occurred in 9 h. By contrast, free cells required 18 h to achieve the same result. Free cells may, therefore, be more prone to toxicity from chromate and other metals as compared to immobilized cells, the latter of which may have some protection from the toxic compounds present in the effluent (Poopal and Laxman 2008).

Saifuddin and Raziah (2007) studied the chromium biosorption potential of chitosan immobilized onto *S. cerevisiae* biomass in aqueous solutions. The relative rates of removal were studied as a function of pH, temperature, biosorbent and chromium concentrations, contact time, agitation speed, interference and reusability. A maximum of 95% chromate removal efficiency (from initial 50 mg Cr^{6+} L^{-1} aqueous solution) was achieved within 45 min under optimized conditions. After chromate adsorption, the adsorbent was regenerated with 0.1 M Tris buffer (pH 7.8). The biomass had good stability with no significant decrease in recoveries of chromium, even after eight runs.

In column studies , Quintelas et al. (2008) observed metal uptake rates of 5.82, 5.35, and 4.12 mg Cr^{6+} g^{-1} biosorbent (from an initial 100 mg Cr^{6+} L^{-1}), respectively, by using granular-activated carbon-supported *S. equisimilis*, *B. coagulans*, and *E. coli* isolates. For this respective order of isolates, the metal uptake rates were 2.33, 1.98, and 3.60 mg Cr^{6+} g^{-1} biosorbent (from an initial concentration of 50 mg Cr^{6+} L^{-1}). Finally, for an initial 10 mg Cr^{6+} L^{-1} level, the metal uptake rates were 0.66, 1.51, and 1.12 mg Cr^{6+} g^{-1} biosorbent. Interestingly, all strains performed comparably well in complex growth media, despite the differences in their cell

wall structure and composition. This may have resulted from the specific chemical reactivity of functional groups (e.g., carboxyl and phosphoryl) that exist within the structural polymers of all bacterial cell walls (Kulczycki et al. 2002). Further, the studies with tannery effluent revealed Cr^{6+} uptake rates of 0.083, 0.090, and 0.110 mg g^{-1} biosorbent (from an initial 4.2 mg total Cr L^{-1}), respectively, for immobilized *S. equisimilus*, *B. coagulans*, and *E. coli* isolates (Quintelas et al. 2008).

Quintelas et al. (2008) also carried out adsorption experiments in batch studies (at initial levels of 50–1,000 mg Cr^{6+} L^{-1}) with fixed doses (10 mg mL^{-1}) of granulated activated carbon coated with biofilms of individual *S. equisimilus*, *B. coagulans*, and *E. coli* isolates. From the initial 50–1,000 mg Cr^{6+} L^{-1} levels present, the percentage of Cr^{6+} removal decreased (46.9–17.2% for the *B. coagulans* biofilm, 36.6–10.8% for the *E. coli* biofilm and 72–46.3%, for the *S. equisimilis* biofilm). These differences in adsorption results from the very high or very low ratios of biosorbent to initial Cr^{6+} concentration (Padmesh et al. 2005). Horsfall et al. (2006) further affirmed that at a higher concentration, the reduced average distance between the adsorbing species affects the charge distribution of its neighbors. This alters the ability of species to migrate onto biomass surface, which, in turn, produces reduced adsorption.

Farag and Zaki (2010) tested Ca-alginate immobilized *Pseudomonas* S4 strain in several consecutive chromium reduction experiments to investigate the possible deactivation of cells with repeated use. The immobilized cells could be reused three times each for 3 days, and could achieve an 80% Cr^{6+} reduction efficiency, without losing chromium reduction activity. Benazir et al. (2010) compared the chromium remediation efficiencies of *B. subtilis*, *P. aeruginosa*, and *S. cerevisiae* in consortia and in their immobilized forms. The best chromium reduction activity was observed for the *S. cerevisiae–P. aeruginosa* consortium, followed by immobilized beads of the *S. cerevisiae* and *B. subtilis–S. cerevisiae* consortia.

9 Simultaneous Bioremediation of Chromium and Phenolics

Cr^{6+}, other heavy metals, and toxic aromatic organic compounds, viz., phenols, naphthalene, trichloroethylene are often discharged to the environment together in industrial effluents (Patterson 1985; Canter 1986; Keely and Boateng 1987; Reid et al. 1994; Wang and Chirwa 2001). Such pollutants may be detrimental to a variety of organisms including humans. Hexavalent chromium and organic pollutants originate from various sources, including leather tanning, dyeing, textile manufacture, photographic film making, wood preservation, car manufacturing, petroleum refining, and agriculture industries (Chirwa and Wang 2000; Aksu and Akpinar 2001; Aksu and Gonen 2006).

In leather tanning, pentachlorophenol (PCP) is used as a biocide (Thakur et al. 2001). Because PCP is recalcitrant, it is usually present in tanning and other industrial discharges (Srivastava and Thakur 2003). Owing to its toxicity, PCP is on the priority pollutants list of the United State's Environmental Protection

Agency. As per Indian Standard Institution (ISI) limits, the permissible level for phenolic compounds in inland surface waters is 0.002 mg L^{-1}, whereas the similar limit in leachates is 1 mg L^{-1}. The European Council Directive has set a limit of 0.5 μg L^{-1} to regulate phenol concentration in drinking water. Phenolic compounds are easily bioaccumulated in various food chains of biological systems, and thus can cause profound problems to the human health. Phenolics also contribute to off-flavor problems in drinking water and in food processing waters (Yang and Humphrey 1975). Phenolic contaminants can damage sensitive cells by permeating cytoplasmic membranes and coagulating cytoplasm. Phenolic compounds are quite inhibitory to environmental microbes, because of their action on membrane function (Copley 2000) and their ability to uncouple oxidative phosphorylation (Ito and Ohnishi 1982). Therefore, wastewater contaminated with Cr^{6+} and phenolics together should be treated carefully before being discharged into receiving water bodies.

Despite widespread pollution caused by PCP, only a few indigenous bacterial strains have been isolated that are capable of degrading it. However, several nonindigenous bacterial strains, such as *Pseudomonas* sp., *S. marcescens*, and *Sphingomonas chlorophenolicum*, are able to degrade PCP (Yang et al. 2006; Singh et al. 2007; Sharma and Thakur 2008). While extensive research has been performed on the uptake of single heavy metal species or toxic organic compounds by microbial cells, little attention has been paid to the bioremediation of binary organic compound–metal ion mixtures. Therefore, treatment strategies that are applicable for a specific metallic pollutant may not work for dual ones, because of the toxicity to the system posed by the organic component(s) or vice versa. Therefore, isolating microbial strains having the potential to degrade PCP and simultaneously bioaccumulating chromium would be valuable for effecting binary-compound bioremediation (Srivastava et al. 2007).

During the last decade, several studies were conducted that were designed to remove organic compounds and/or Cr^{6+} using fungi (Choi et al. 2002; Denizil et al. 2004; Taseli and Gokcay 2005), yeast (Hamed et al. 2004), microalgae (Lima et al. 2004), bacteria (Edgehill 1996; Quintelas and Tavares 2001; Quintelas and Tavares 2002), bacterial consortia (Chirwa and Wang 2000), anaerobic activated sludge (Aksu and Akpinar 2001), among others. However, only limited research was performed on native microbes for the purpose of simultaneous bioremediation of Cr^{6+} and phenolics in tannery effluent (Chirwa and Wang 2005; Srivastava et al. 2007; Tziotzios et al. 2008).

In Table 4, we summarize experimental findings from studies in which simultaneous bioremediation of Cr^{6+} and phenolic compounds from tannery effluent were addressed under optimized cultural and nutritional conditions. This research has addressed many aspects of the simultaneous remediation of phenolics and Cr^{6+}, including what constitutes suitable consortia/cocultures of microorganisms for degrading such binary mixtures (Wang and Chirwa 1998; Chirwa and Wang 2000; Wang and Chirwa 2001; Aksu and Gonen 2006; Quintelas et al. 2006; Liu et al. 2008; Tziotzios et al. 2008). In most of these studies, phenol was used as a carbon and energy source. Bacteria capable of reducing Cr^{6+} utilized organic acid by-products of phenol's degradation to effect microbial growth and Cr^{6+} reduction.

Table 4 Simultaneous microbial remediation of hexavalent chromium and phenolic compounds under various optimized experimental conditions

		Experimental conditions									
Microorganisms	Source of organism	System (medium/ effluent)	Initial Cr^{6+} concentration (mg L^{-1})	Initial phenol/ chlorophenols (mg L^{-1})	Inoculum size (%v/v)/ cfu mL^{-1}	pH	Temperature (°C)	Time (h)	Chromium removal (%)	Phenol/ chlorophenol (% removal)	Reference
Escherichia coli ATCC 33456 +Phenol degraders	Culture collection (ATCC)	Basal mineral medium	4.41	253	2.5×10^7	7.0	35	67	~100	~100	Chirwa and Wang (2000)
Mowital®B30H resin immobilized activated sludge Biomass	Waste activated sludge	Continuous Fixed bed column (effluent)	503.1	502.8	3 g of immobilized beads containing 1.5 g of dried activated sludge biomass	1.0	25		8.9 (8.0 mg adsorbed g^{-1} dried activated sludge)	6.5 (2.1 mg adsorbed g^{-1} dried activated sludge)	Aksu and Gonen (2006)
Arthrobacter viscosus	Culture collection (STCC)	Minicolumn (metal and organic solutions)	60	100	Culture biofilm on granulated activated carbon	<8	–	15	11.3	7	Quintelas et al. (2006)
Acinetobacter sp. followed by *Aspergillus niger*	Tannery effluent	Sequential bioreactor (tannery effluent)	557	15	–	7.0	–	360	90	67	Srivastava et al. (2007)

Mixed bacterial culture containing Cr^{6+}-reducing and phenol degrading bacteria	Olive pulp and industrial sludge	Packed bed bioreactor (effluent)	5.5	350	Mixed inoculum (50 ml L^{-1} of the bulk liquid) of enriched bacteria	7.3	26 ± 1	24	0.062 g Cr^{6+} L^{-1} d^{-1}	3.543 g phenol L^{-1} d^{-1}	Tziotzios et al. (2008)
Pseudomonas aeruginosa CCTCC AB91095	Culture collection (CCTCC)	Shake flask (mineral liquid medium)	5	100	2	7.0	37	72	~100	98±1	Song et al. (2009)
Pseudomonas putida + a mixed cultures of *Bacillus cereus*, *Bacillus thuringiensis*, *Bacillus mycoides* and serovar *finitimus*	Activated sludge	Trickle bed reactor (effluent)	10	80	–	–	26–30	–	70	80	Chirwa and Smit (2010)

Shen and Wang (1995) observed simultaneous reduction of Cr^{6+} and degradation of phenol in a defined coculture of a Cr^{6+} reducer, *E. coli* ATCC33456, and a phenol degrader, *P. putida* DMP-1. They demonstrated that *E. coli* was unable to utilize phenol as a growth substrate. However, when *E. coli* was cocultured with *P. putida* (with phenol as a sole carbon source in the medium), *E. coli* exhibited a growth curve characteristic similar to that of *P. putida*. Further, Cr^{6+} reduction occurred as long as phenol was present, and ceased upon its depletion. These phenomena were reproduced by spiking phenol at 12 and 26 h of the incubation. Since *P. putida* supported the growth of *E. coli* on phenol, the growth substrate may have served as an electron donor for *E. coli* to reduce Cr^{6+}.

Shen and Wang (1995) asserted that both the rate and extent of Cr^{6+} reduction and phenol degradation were significantly influenced by the composition of the coculture population. Although Cr^{6+} reduction occurred as a result of *E. coli* metabolism, the rate of phenol degradation by *P. putida* may have become a rate-limiting factor for Cr^{6+} reduction at a low population ratio of *P. putida* to *E. coli*. Further, the phenol degradation by *P. putida* was highly susceptible to the presence of Cr^{6+}, whereas Cr^{6+} reduction by *E. coli* was significantly influenced by phenol only at >9 mM concentration . Interestingly, besides phenol, several aromatic compounds, including 2-chlorophenol, *p*-cresol, 2,6-dimethylphenol, 3,5-dimethylphenol, 3,4-dimethylphenol, benzene, and toluene, were also utilized as electron donors for the reduction of Cr^{6+}. The ability of a coculture to utilize a wide range of aromatic pollutants for the reduction of Cr^{6+} illustrated the potential for simultaneous detoxification of mixtures of chromium and various aromatic contaminants (Shen and Wang 1995).

Chirwa and Wang (2000) also studied the simultaneous bioremoval of Cr^{6+} and phenol in an anaerobic phenol-degrading bacterial consortium that was cocultured with a *E. coli* ATCC33456 strain capable of reducing Cr^{6+}. Optimum Cr^{6+} reduction was observed at initial levels of 200 mg phenol L^{-1} and 2 mg Cr^{6+} L^{-1}, while complete phenol degradation was evident only at a low initial Cr^{6+} concentrations ($\leq$10 mg L^{-1}). Further, *E. coli* utilized metabolites of phenol degradation as electron donors for the reduction of Cr^{6+}. Elizabeth and Esther (2004) studied the use of *Bacillus s*pecies (BS2) and *S. aureus* as biosorbents for Cr^{6+} remediation and phenol degradation. The bioremediation rate was 100% for Cr^{6+} and 8% for phenol.

A biofilm is composed of bacterial cells, extracellular polymers produced by bacteria, lysis and hydrolysis products, attached matter and some inorganic compounds. In a biofilm, the possible sorption sites are extracellular polymeric substances, cell walls, cell membranes, and the cytoplasm (Aksu 2005). Chirwa and Wang (2001) studied bioremediation of phenol and Cr^{6+} in a continuous-flow fixed-film phenol degrader (*P. putida* DMP-1), and a Cr^{6+}-reducer (*E. coli* ATCC33456). Complete Cr^{6+} reduction and phenol degradation occurred at loadings of 5–21 mg Cr^{6+} $L^{-1}d^{-1}$ and 840–3,350 mg phenol L^{-1} d^{-1}. Moreover, a mathematical model developed by the same authors (Chirwa and Wang 2005) indicated that removal of inert cells was critical for the bioreactor's proper operation. The model was suitable for simultaneous Cr^{6+} removal and phenol degradation under loading levels of 2.5–3.5 mg Cr^{6+} $L^{-1}d^{-1}$ and 50–850 mg PCP L^{-1} d^{-1}, respectively.

Aksu and Gonen (2006) studied the simultaneous bioremediation of phenol and Cr^{6+} (at initial concentrations ranging 50–500 mg L^{-1} for both the pollutants) by Mowital B3OH resin-immobilized activated sludge held at a pH of 1.0, and with a flow rate of 0.8 mL min^{-1} in a packed-bed column bioreactor. The bioremoval process was successful only at the lower concentrations of both the pollutants. Tziotzios et al. (2008) used a pilot-scale packed-bed bioreactor for simultaneous phenol degradation and Cr^{6+} reduction, using a new culture consortium of Cr^{6+}-reducing and phenol-degrading bacteria. Phenol inhibited the reduction of Cr^{6+}. Conversely, elevated Cr^{6+} concentrations did not affect phenol's degradation. Quintelas et al. (2006) used a biofilm of *A. viscosus* supported on granular activated carbon for single and dual remediation of Cr^{6+} and organic compounds (chlorophenol, phenol, and *o*-cresol) from aqueous solutions. The authors observed a maximum Cr^{6+} adsorption of 11.3% at initial phenol and Cr^{6+} concentrations of 100 and 60 mg L^{-1}, respectively. However, the maximum phenol degradation of 63% was evident at levels of 10 mg phenol L^{-1} and 60 mg Cr^{6+} L^{-1}. Chirwa and Smit (2010) attempted simultaneous Cr^{6+} reduction and phenol degradation in a trickle-bed reactor system by using a mixed culture isolated from activated sludge from the Brits Wastewater Treatment Works (NW Province, South Africa). The reactor achieved 70% Cr^{6+} removal, and 80% degradation of phenol (from initial levels of 10 mg Cr^{6+} L^{-1} and 80 mg phenol L^{-1}), under optimum operational conditions.

Srivastava et al. (2007) employed a fungal strain (viz., *A. niger* FK1) and a bacterial isolate (viz., *Acinetobacter* sp.) individually for bioremediation of chromium and PCP in a sequential bioreactor fabricated by Shah and Thakur (2002). The tannery effluent treated in set-1, initially with *Acinetobacter* sp., followed with fungus treatment, respectively, remediated 90% of Cr^{6+} (from an initial 557 ppm level of chromium) and 67% of PCP (from an initial 15 ppm PCP level) in 15 days. In the set-2 sequential bioreactor, wherein the effluent was first treated by the fungus and then by the bacteria, removed only 64.7% and 58% of chromium and PCP, respectively, within 15 days. The higher level of chromium removal in the set-1 bioreactor was attributed to the utilization of PCP as a food source in step-1 by *Acinetobacter* sp., thereby exerting no inhibitory effect of PCP on fungus for removing Cr^{6+} in step-2 (Srivastava et al. 2007). However in the set-2 bioreactor, the growth of the fungus was inhibited by PCP in step-1, thereby decreasing the extent of chromium removal, which led to bioaccumulation of Cr^{6+} in the fungal mycelium (Srivastava et al. 2007). The fungal biomass contains a relatively high percentage of cell wall material, which serves as an excellent metal binder. However, most fungi do not have the ability to degrade chlorinated organic compounds (Edgehill and Finn 1983; Kapoor and Viraraghavan 1995; McLean et al. 2000).

Tripathi et al. (2011b) suggested that the nutritional requirement, growth, and maintenance of an organism can be more conveniently managed if a single microbial strain is employed instead of a coculture or consortia of microorganisms for simultaneous bioremediation of pollutants from the environment. Further, the isolation and selection of indigenous bacterial strains from natural habitats may have the advantage of minimizing inhibitory effects from other toxic pollutants that augment Cr^{6+} in the polluted environment. To have successfully existed where they are found,

native microorganisms must have developed at least some degree of resistance to these toxic compounds.

Some researchers have reported simultaneous bioremediation of Cr^{6+} and aromatic pollutant(s) by a single pure culture of *P. aeruginosa* (Chen et al. 2003; Xu and Liu 2005). Song et al. (2009) attempted simultaneous Cr^{6+} reduction and phenol degradation in a bioreactor by a pure culture of *P. aeruginosa* CCTCC AB91095. The organism utilized phenol as a sole carbon source, possibly through organic acid production. Cr^{6+} inhibited its own reduction as well as phenol degradation at a concentration of >20 mg Cr^{6+} L^{-1}. In contrast, phenol enhanced both the reduction of Cr^{6+} as well as its own degradation at <100 mg PCP L^{-1}. These authors also reported a profound influence of the inoculum dose on both reduction of Cr^{6+} and phenol degradation. The concentrations of Cr^{6+} and PCP declined in 12 h, respectively from 20 to 3.36 mg Cr^{6+} L^{-1} and 100–29.51 mg phenol L^{-1} in cultures containing 5% (v/v) inoculum of *P. aeruginosa*. The trivalent chromium (Cr^{3+}) was the predominant product of the reduction of Cr^{6+}. Chirwa and Wang (2000) reported that phenol (at a concentration exceeding the optimum level) inhibited the reduction of Cr^{6+} and its own degradation.

Mathew et al. (2010) studied the simultaneous bioremediation of Cr^{6+} (at initial levels of 10–50 ppm) and phenol (at initial levels of 50 and 100 ppm) by five individual bacterial cultures, viz., species of *Pseudomonas*, *Amphibacillus*, *Micrococcus, Moraxella,* and *E. coli* which were isolated from the retting grounds of Kadinamkulum backwaters. Contrary to the findings of Shen and Wang (1995), these authors observed no inhibitory effect of Cr^{6+} on phenol degradation for any of the test cultures. Moreover, the extent of Cr^{6+} reduction was similar at both concentrations of phenol tested. Shen and Wang (1995) tested cocultures of *E. coli* and *P. putida,* and reported that the degradation of phenol by *P. putida* was inhibited by Cr^{6+}, which increased as the Cr^{6+} concentration increased.

Tripathi and coworker(s) (2010, 2011b) isolated, for the first time, a native bacterial strain RMLAU1 of *B. cereus* from treated tannery effluent that was tolerant to 500 mg PCP L^{-1} and 200 mg Cr^{6+} L^{-1}. The isolate degraded 56.5% PCP (from the initial 500 mg L^{-1} level) and simultaneously bioremediated 74.5% chromium (from the initial 200 mg L^{-1} level). The uptake rate was 40.6 mg g^{-1} biomass under optimized conditions of pH 7.0, 35°C, 1.0% (v/v) inoculum and 150 rpm, within 48 h of incubation. Results of the above studies indicated the ability of a single organism to successfully perform both Cr^{6+} reduction and phenol degradation that had formerly been performed only by mixed cultures.

10 Conclusions

After reviewing the literature on the potential for bioremediation to address chromium pollution in tannery waste, we have concluded that:

1. Current techniques to remediate Cr pollution from tanneries are insufficient, not always environmentally responsible, and usually generate unacceptable levels of secondary pollution, e.g., sludges.

2. Bioremediation, although far from technologically mature, offers a real, and practicable alternative as means to clean Cr, not only from tannery waste, but also to clean many heavy metals from several industrial effluent types.
3. Four types of bioremedial processes, at present, seem particularly applicable for treating tannery waste, viz., (a) biosorption, (b) bioaccumulation, (c) bioreduction, and (d) immobilization of microbial cells for bioremediation.
4. Simultaneous bioremediation of heavy metals including Cr^{6+}, and certain chlorinated phenolics would be a more viable option for sufficiently cleaning wastewaters of various toxic constituents hazardous to human health and to the environment.

11 Summary

Bioremediation offers the possibility of using living organisms (bacteria, fungi, algae, or plants), but primarily microorganisms, to degrade or remove environmental contaminants, and transform them into nontoxic or less-toxic forms. The major advantages of bioremediation over conventional physicochemical and biological treatment methods include low cost, good efficiency, minimization of chemicals, reduced quantity of secondary sludge, regeneration of cell biomass, and the possibility of recovering pollutant metals. Leather industries, which extensively employ chromium compounds in the tanning process, discharge spent-chromium-laden effluent into nearby water bodies. Worldwide, chromium is known to be one of the most common inorganic contaminants of groundwater at pollutant hazardous sites. Hexavalent chromium poses a health risk to all forms of life. Bioremediation of chromium extant in tannery waste involves different strategies that include biosorption, bioaccumulation, bioreduction, and immobilization of biomaterial(s). Biosorption is a nondirected physiochemical interaction that occurs between metal species and the cellular components of biological species. It is metabolism-dependent when living biomass is employed, and metabolism-independent in dead cell biomass. Dead cell biomass is much more effective than living cell biomass at biosorping heavy metals, including chromium. Bioaccumulation is a metabolically active process in living organisms that works through adsorption, intracellular accumulation, and bioprecipitation mechanisms. In bioreduction processes, microorganisms alter the oxidation/reduction state of toxic metals through direct or indirect biological and chemical process(es). Bioreduction of Cr^{6+} to Cr^{3+} not only decreases the chromium toxicity to living organisms, but also helps precipitate chromium at a neutral pH for further physical removal, thus offering promise as a bioremediation strategy. However, biosorption, bioaccumulation, and bioreduction methods that rely on free cells for bioremediation suffer from Cr^{6+} toxicity, and cell damage. Therefore, immobilization of microbial cell biomass enhances bioremediation and renders industrial bioremediation processes more economically viable from reduced free-cells toxicity, easier separation of biosorbents from the tannery effluent, ability to achieve multiple biosorption cycles, and desorption (elution) of metal(s) from matrices for reuse. Thus, microbial bioremediation can be a cost competitive strategy and beneficial bioresource for removing many hazardous contaminants from tannery and other industrial wastes.

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Index

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