

Review

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Nomenclature:

Acetochlor; α -chloroacetamides; α -oxyacetamides; α -thioacetamides; azolyl-carboxamides; benzofurans; fenoxasulfone; isoxazolines; thiocarbamates; oxiranes; S-metolachlor; pyroxasulfone; Palmer amaranth, *Amaranthus palmeri* S. Watson; waterhemp, *Amaranthus tuberculatus* (Moq) J.D. Sauer; barley, *Hordeum vulgare* L.; corn, *Zea mays* L.; oat, *Avena sativa* L.; sorghum, *Sorghum bicolor* (L.) Moench; soybean, *Glycine max* L.; sugarcane, *Saccharum officinarum* L.; wheat, *Triticum aestivum* L.

Keywords:

α -chloroacetamides; Group 15 herbicides; isoxazolines; residual herbicides; shoot and root tissue; resistant weeds; weed management




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Very long chain fatty acid-inhibiting herbicides: Current uses, site of action, herbicide-resistant weeds, and future

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Abstract

The herbicides that inhibit very-long-chain fatty acid (VLCFA) elongases are primarily used for residual weed control in corn, barley, oat, sorghum, soybean, sugarcane, certain vegetable crops, and wheat production fields in the United States. They act primarily by inhibiting shoot development of susceptible species, preventing weed emergence and growth. The objectives of this review were to summarize 1) the chemical family of VLCFA-inhibiting herbicides and their use in the United States, 2) the VLCFA biosynthesis in plants and their site of action, 3) VLCFA-inhibitor resistant weeds and their mechanism of resistance, and 4) the future of VLCFA-inhibiting herbicides. After their reclassification as Group 15 herbicides to include shoot growth-inhibiting herbicides (Group 8), the VLCFA-inhibiting herbicides are currently represented by eight chemical families (benzofurans, thiocarbamates, α -chloroacetamides, α -oxyacetamides, azolyl-carboxamides, isoxazolines, α -thioacetamides, and oxiranes). On average, VLCFA-inhibiting herbicides are applied once a year to both corn and soybean crops in the United States with acetochlor and S-metolachlor being the most used VLCFA-inhibiting herbicides in corn and soybean production, respectively. The site of action of Group 15 herbicides results from inhibition of the VLCFA synthase, which is encoded by several fatty acid elongase (*FAE1*)-like genes in VLCFA elongase complex in an endoplasmic reticulum. The VLCFA synthase is a condensing enzyme, and relies on a conserved, reactive cysteinyl sulfur in its active site that performs a nucleophilic attack on either the natural substrate (fatty acyl-CoA) or the herbicide. As of August 2023, 13 weed species have been documented to be resistant to VLCFA inhibitors, including 11 monocot weeds and two dicot weeds (Palmer amaranth and waterhemp). The isoxazolines (pyroxasulfone and fenoxasulfone) are the most recently (2014) discovered VLCFA-inhibiting herbicides. Although the intensity of VLCFA-inhibitor-directed discovery efforts has decreased over the past decade, this biochemical pathway remains a viable mechanistic target for the discovery of herbicide premixes and a valuable component of them.

Introduction

Herbicides that inhibit very-long-chain fatty acids (VLCFAs) have been in use for more than 60 yr for broad-spectrum control of weeds in several crops (Senseman 2007). S-ethyl dipropylthiocarbamate (EPTC) was the first VLCFA inhibitor developed by Stauffer Chemical Company in 1958, while the most recent discoveries were pyroxasulfone and fenoxasulfone, in 2014 (Table 1). These herbicides inhibit elongases after the formation of malonyl-Co-A in the fatty acid biosynthesis pathway. The VLCFA-inhibiting herbicides are typically applied on the soil before weed emergence (Jhala et al. 2015), and upon plant uptake, the compound is metabolically cleaved to produce an active form of the herbicide via sulfoxide enzyme activity (Sherwani et al. 2015).

The VLCFA-inhibiting herbicides can be applied postemergence (POST) to several crops and provides overlapping residual activity. It can be used throughout the autumn and early preplant to early POST periods for controlling weeds, including those that are resistant to glyphosate, acetolactate synthase (ALS) inhibitor, acetyl CoA carboxylase (ACCase) inhibitor,



Table 1. Registration timeline of selected very-long-chain fatty acid-inhibiting herbicides in the United States according to the U.S. Environmental Protection Agency.^a

Common name	Chemical family	Discovered by	Year
EPTC	Thiocarbamate	Stauffer Chemical Company	1958
Triallate	Thiocarbamate	Gowan	1961
Propachlor	α -chloroacetamides	Monsanto	1964
Vernolate	Thiocarbamate	Zeneca	1964
Butylate	Thiocarbamate	Zeneca	1967
Cycloate	Thiocarbamate	Stauffer Chemical Company	1967
Alachlor	α -chloroacetamides	Monsanto	1969
Metolachlor	α -chloroacetamides	Syngenta	1976
Ethofumesate	Benzofurans	Bayer CropScience and United Phosphorous	1977
Thiobencarb	Thiocarbamate	K-I Chemical Research Institute	1982
Dimethenamid	α -chloroacetamides	BASF	1989
Acetochlor	α -chloroacetamides	Monsanto	1994
Flufenacet	α -oxyacetamides	Bayer	1998
Pyroxasulfone	Isoxazolines	K-I Chemical Research Institute, Kumiai Chemical Industry, and Ihara Chemical Industry	2014
Fenoxasulfone	Isoxazolines	Kumiai Chemical Industry	2014

^aSee <https://www.epa.gov/>.

and triazines (Shaner et al. 2014). In susceptible grass and broadleaf weeds, the growth of the apical meristem and coleoptile is disrupted after germination, resulting in a failure to emerge. When susceptible monocots do emerge, they appear twisted and malformed, with leaves tightly rolled in a whorl and unable to unroll normally. Broadleaf seedlings may exhibit slightly cupped or crinkled leaves, along with shortened leaf midribs, resulting in a drawstring effect on the leaf tip (Shaner et al. 2014).

The VLCFA-inhibiting herbicides are classified as Group 15 herbicides by the Weed Science Society of America (WSSA) and the Herbicide Resistance Action Committee (Mallory-Smith and Retzinger 2017). These herbicides inhibit fatty acid synthesis in plants, an important component of plant cell membranes, waxes, and cuticles, and they play a crucial role in plant growth and development (Millar and Kunst 1997; Post-Beittenmiller 1996). The VLCFA-inhibiting herbicides are sometimes referred to as shoot-growth-inhibiting herbicides and are commonly applied to soil to control seedling grasses and certain broadleaf weeds as they emerge, and to suppress some perennial weed species that arise from tubers and rhizomes. The VLCFA-inhibiting herbicides are primarily absorbed by plant roots and are translocated to the shoot and leaf tissues primarily via xylem (Fuerst 1987), in addition to being absorbed to a lesser extent through the shoots and leaves of the plant.

The objectives of this review were to 1) summarize the chemical families of VLCFA-inhibiting herbicides and their use in the United States; 2) provide an overview of VLCFA biosynthesis in plants, VLCFA profiles in Group 15 herbicide-treated plants, and inhibition of VLCFA elongases by Group 15 herbicides; 3) describe VLCFA-inhibiting herbicide-resistant weeds and their mechanism of resistance; and 4) explore the future of VLCFA-inhibiting herbicides.

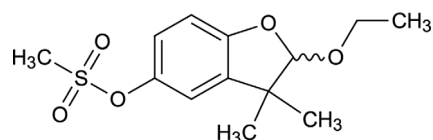
Chemical Families of VLCFA-Inhibiting Herbicides

Herbicides that inhibit shoot growth were classified into Group 8 (lipid synthesis-inhibitor) and Group 15 (VLCFA-inhibitor);

Table 2. Very-long-chain fatty acid-inhibiting herbicide chemical families and active ingredients according to the Weed Science Society of America and the Herbicide Resistance Action Committee classification list.^a

Chemical family	Common names
α -Chloroacetamides	Acetochlor, alachlor, allidochlor=CDA, butachlor, butenachlor, delachlor, diethyl-ethyl, dimethachlor, dimethenamid, metazachlor, pethoxamid, pretilachlor, propachlor, propisochlor, prynachlor, metolachlor, and therylchlor.
α -Oxyacetamides	Mefenacet and flufenacet
α -Thioacetamides	Anilofos and piperophos
Azoly-carboxamides	Fentrazamide and ipfencarbazone
Benzofurans	Ethofumesate and benfuresate
Isoxazolines	Pyroxasulfone and fenoxasulfone
Oxiranes	Indanofana and tridiphane
Thiocarbamate	Butylate, cycloate, dimepiperate, EPTC, esprocarb, molinate, orbencarb, pebulate, prosulfocarb, thiobencarb (syn. benthocarb), tiocarbazil, triallate, and vernolate.

^aSee <https://wssa.net/wssa/weed/herbicides/> (last modified May 5, 2021).

**Figure 1.** Chemical structure of ethofumesate, which inhibits very-long-chain fatty acids.

however, the Group 8 herbicides were later reclassified into Group 15 by the WSSA Herbicide Resistance Action Committee (HRAC) in 2021 (Table 2). Napropamide belonged to the acetamide family of Group 15 in the previous herbicide classification; however, it is classified as a Group 0 herbicide in the revised classification in 2021 (WSSA 2021). The reclassification of VLCFA-inhibiting herbicides reflects the understanding of their site of action (SoA). The VLCFA-inhibiting herbicides are currently represented by eight chemical families: α -chloroacetamides, α -oxyacetamides, α -thioacetamides, azoly-carboxamides, benzofurans, isoxazolines, oxiranes, and thiocarbamates (Table 2). They are known to inhibit various biochemical pathways in plants, including the biosynthesis of fatty acids and lipids (reducing cuticular wax deposition), proteins (isoprenoids, and flavonoids), and gibberellins (due to kaurene synthesis inhibition) (Fuerst 1987; WSSA 2021). The chemical families of VLCFA-inhibiting herbicides are briefly discussed below.

Benzofurans

Benzofurans and thiocarbamates were chemical families of Group 8 that were reclassified into Group 15. Benzofurans are a family of shoot-inhibiting herbicides that act by inhibiting meristem growth, cell division, and the formation of cuticles, ultimately leading to the retardation of plant growth (Kohler and Branham 2002; Székács 2021). Ethofumesate, an active ingredient within this family (Figure 1), is commonly used preemergence (PRE) in the United States to control weeds and POST to selectively control annual grasses and broadleaf weeds in crops that include sugarbeet (*Beta vulgaris* L.), vegetables, and grass seed production (Abulnaja et al. 1992; Albuquerque et al. 2018). Moreover, ethofumesate reduces the formation of epicuticular wax on leaves (Abulnaja et al. 1992).

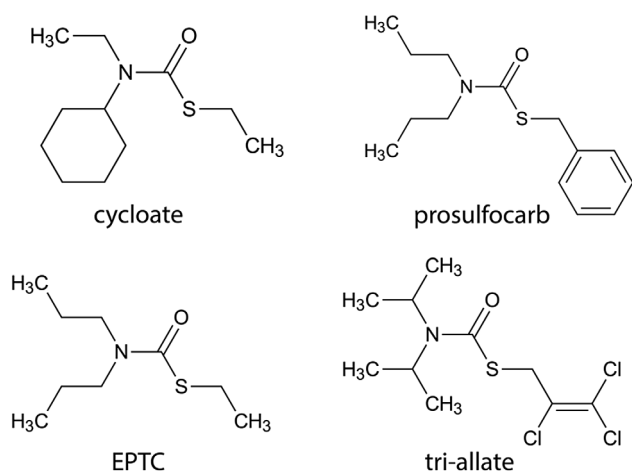


Figure 2. Chemical structures of some thiocarbamate herbicides.

It has limited mobility, with little leaching occurring in soils with greater than 1% organic matter because the herbicide remains relatively close to the application site (Shaner et al. 2014).

Thiocarbamates

Thiocarbamates are commonly applied preplant incorporated (PPI) or POST for residual control of annual grasses such as foxtail (*Setaria* spp.), barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv], fall panicum (*Panicum dichotomiflorum* Michx), johnsongrass [*Sorghum halepense* (L.) Pers], shattercane (*Sorghum bicolor* L.), nutsedge (*Cyperus* spp.), and some broadleaf weeds in crops of wheat, barley, pulses, sugarbeet, corn, soybean, cotton, dry bean (*Phaseolus vulgaris* L.), and others (Fuerst 1987; Shaner et al. 2014). The active ingredients of thiocarbamates are characterized by the presence of a thiocarbamate group [-S-C(=O)-NR₂] in their chemical structure (Figure 2), which is responsible for their herbicidal activity (WHO 1988). Some examples of thiocarbamate herbicides include butylate, cycloate, EPTC, molinate, prosulfocarb, thiobencarb, and triallate (Table 2; Figure 2). Thiocarbamates are generally not mobile in soils with high organic matter content, but can be subject to volatilization losses when applied to warm soils and not properly incorporated (Shaner et al. 2014). Wilson (1984) reported accelerated degradation of thiocarbamate herbicides in soil with prior thiocarbamate herbicide exposure due to buildup of microbes that break them down.

α -Chloroacetamides

Chloroacetamide herbicides are widely used to control annual grasses and small-seeded broadleaf weeds in a variety of crops including corn, soybean, peanut (*Arachis hypogaea* L.), cotton, sugarbeet, and solanaceous vegetables. These herbicides can be applied to crops at various stages of plant growth, such as early preplant, PRE, or early POST to provide residual weed control. The α -chloroacetamide herbicides include acetochlor, alachlor, butachlor, dimethenamid-*P*, pretilachlor, and metolachlor (Figure 3).

Metolachlor is often used as a residual herbicide, and contains four isomers that can be classified into *S*- and *R*-metolachlor isomers (O'Connell et al. 1998). The effectiveness of metolachlor is dependent on the concentration of isomers, with the *S*-isomers being more effective than the *R*-isomers (O'Connell et al. 1998; Shaner et al. 2006). The application rate of *S*-metolachlor is 35%

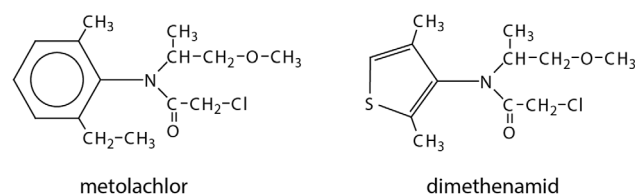


Figure 3. Chemical structures of some α -chloroacetamides.

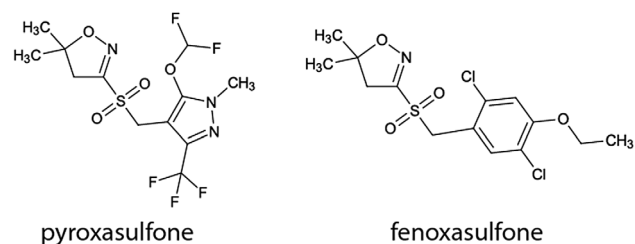


Figure 4. Chemical structures of isoxazolines.

lower than the racemic mixture of metolachlor on an active ingredient basis with residues usually not persisting long enough to affect crops in the following season (Shaner et al. 2014). Dimethenamid-*P* is another example of an α -chloroacetamide herbicide widely used for residual control of annual grasses and certain annual broadleaf weeds in crops such as corn, soybean, dry bean, peanut, and sorghum (Figure 3). It is primarily absorbed by emerging shoots and roots and has a half-life of approximately 20 d (Shaner et al. 2014).

Isoxazolines

Pyroxasulfone and fenoxasulfone belong to the isoxazoline chemical family of VLCFA-inhibiting herbicides (Figure 4; WSSA 2021). Pyroxasulfone is a residual herbicide that is effective for controlling grass and broadleaf weeds while providing selectivity in crops such as corn, wheat, and soybean (Tanetani et al. 2009). Pyroxasulfone is absorbed primarily by emerging shoots and roots and is translocated acropetally throughout the shoots (Shaner et al. 2014). It is moderately adsorbed to soil and has low leaching potential. It is less mobile in fine and medium-textured soils and more mobile in coarse-textured soils. It is not persistent in soils and has a terrestrial field dissipation half-life of 16 to 26 d. Negligible losses occur due to photodegradation or volatilization, and microbial degradation is a major contributor to field dissipation (Shaner et al. 2014). Fenoxasulfone is a PRE herbicide that is effective for controlling annual broadleaf and grass weeds in paddy-field rice (*Oryza sativa* L.) cultivation (Tanetani et al. 2011a). It has long residual activity and displays herbicidal activity against *Echinochloa* spp. And other annual weeds at 150 to 200 g ai ha⁻¹ (Fujinami et al. 2019; Umetsu and Shirai 2020).

A-Oxyacetamides

The most common oxyacetamide herbicides are mefenacet and flufenacet. Mefenacet is primarily used for weed control in transplanted rice and is not currently marketed in the United States (Fedtke 1991; Shaner et al. 2014). Flufenacet has been mixed with a relatively low rate of metribuzin (AXIOM™; Bayer CropScience, St Louis, MO), or with isoxaflutole and thiencazone-methyl (TriVolt™; Bayer CropScience), a corn herbicide. Flufenacet

controls most annual grasses and certain small-seeded broadleaf weeds. Flufenacet has a short to moderate persistence, with a half-life of 29 d in loamy sand soil with 0.9% organic matter and pH 5.7; and a half-life of 62 d on loamy sand with 0.5% organic matter, and pH 5.6. In most soil textures, oxyacetamides have low to moderate mobility and are primarily degraded by microbial activity (Shaner et al. 2014).

Azoly-Carboxamides

Ipfencarbazone-methyl, fentrazamide, and their related compounds are VLCFA-inhibiting herbicides that belong to the azoly-carboxamides chemical family. Ipfencarbazone is a PRE and early POST herbicide used in rice production with high efficacy and long residual activity against a broad range of weeds, including annual grasses, sedges, and some broadleaf weeds (Kasahara et al. 2019; Kido et al. 2016; Umetsu and Shirai 2020). Fentrazamide is a selective herbicide widely used to control *Echinochloa* spp. And annual sedges in rice. At a rate of 200 to 300 g ai ha⁻¹, it has shown efficacy against *Echinochloa* spp. Within a wide range of growth stages, from PRE up to the 3-leaf stage of the weed in rice (Shaner et al. 2014). Fentrazamide has low mobility in Japanese paddy soils, and is absorbed through the roots and shoots of susceptible plants and dissipates rapidly in water with a half-life of soil metabolism of about 30 d in volcanic soil and 20 d in alluvial soil (Shaner et al. 2014).

A-Thioacetamides

Thioacetamides are a chemical family of VLCFA-inhibiting herbicides, with anilofos and piperophos being examples in this class. Anilofos is a PRE and early POST herbicide used to control annual grasses, sedges, and selected broadleaf weeds in transplanted and direct-seeded rice at a rate of 300 to 450 g ai ha⁻¹. It is taken up through the roots and, to some degree, through the leaves, and has an inhibitory effect on shoot and root growth. In soil, anilofos exhibits typical degradation behavior for phosphoric acid, leading to the formation of chloroaniline and CO₂ as end products. This herbicide has a field dissipation DT₅₀ of 30 to 5 d and shows low mobility in soil (Shaner et al. 2014).

Oxiranes

Tridiphane and indanofan belong to the oxirane family (Figure 5). Tridiphane has a synergistic effect on atrazine for controlling grass species such as giant foxtail (*Setaria faberi* Herrm.) because it inhibits glutathione S-transferase (GST) and prevents the detoxification of atrazine (Lamoureux and Rusness 1986). Tridiphane has five chlorines (Figure 5), which can have adverse environmental effects. To overcome this, researchers have developed indanofan, an oxirane herbicide that has less chlorine (Figure 5) and shows relatively better environmental properties (Takahashi et al. 2002). Indanofan at 100 to 150 g ai ha⁻¹ provided complete control of *Echinochloa oryzicola* (Takahashi et al. 2002).

Overview of VLCFA Biosynthesis in Plants

The VLCFAs are essential to plant growth, development, and defense. VLCFAs are synthesized from plastid-derived 16 or 18 carbon long-chain fatty acids (saturated and unsaturated) that are elongated to 30 carbons or more through sequential additions of two carbon atoms by an endoplasmic reticulum (ER)-bound, multienzyme acyl-CoA elongase complex using the substrates fatty

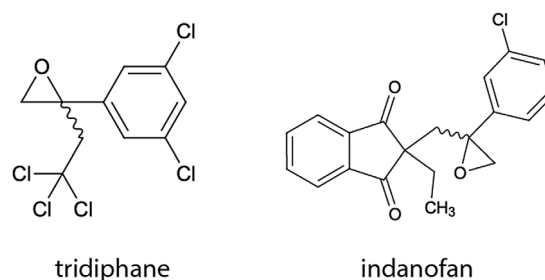


Figure 5. Chemical structures of the oxirane herbicides tridiphane and indanofan.

acyl-CoA and malonyl-CoA (Haslam and Kunst 2013). The elongase complex in the ER contains separate enzymes with at least four distinct, sequential functions: condensation, reduction, dehydration, and a second reduction (Böger et al. 2000; Krähmer et al. 2019). The condensing enzyme (also called VLCFA synthase or 3-ketoacyl-CoA synthase) catalyzes the first, rate-limiting step of these sequential reactions and determines substrate and tissue specificities of VLCFA elongation (Bach and Faure 2010; Millar and Kunst 1997). By contrast, the other three enzymes are constitutively expressed, exhibit broad substrate specificity, and are common to all tissues with VLCFA biosynthetic capacity (Krähmer et al. 2019).

The VLCFA-inhibiting herbicides were discovered and developed in the 1950s (Hamm 1974), but their mode of action (MoA) remained elusive for decades until it was determined that depletion of VLCFA results in phytotoxicity (reviewed by Böger 2003). Condensing enzymes are the SoAs for Group 15 herbicides in plants (Krähmer et al. 2019); their discovery and characterization has led to a considerable number of publications during the past 30 yr to describe their structures, sequences, physiological functions, and roles in determining Group 15 herbicide SoAs. The VLCFAs are a diverse group of nonpolar compounds and vary in their degrees of unsaturation, function, and structure. They are found in lipid seed reserves and signaling molecules, and comprise the main components of cellular membranes, suberin, cuticle waxes, and the outer coats of pollen grains or “tryphine” layer (Bach and Faure 2010; Haslem and Kunst 2013; Millar et al. 1999). Physiological studies found that lipid synthesis and formation of cuticle waxes were inhibited by chloroacetamide herbicides (reviewed by Böger et al. 2000; Fuerst 1987), and studies by Weisshaar and Böger (1987) eventually led to the discovery of the SoA of chloroacetamides. The first clue was a lack of plasma membrane formation, and it was concluded that chloroacetamides inhibit elongation of C16 or C18 long-chain fatty acids into VLCFA at low (micromolar) concentrations (Weisshaar and Böger 1987). Plants deficient in VLCFA have unstable cells that eventually deteriorate, which inhibits growth in meristematic areas and, ultimately, the death of sensitive plant seedlings (Böger 2003; Matthes et al. 1998).

Inhibition of VLCFA Elongases by Group 15 Herbicides

The SoA of Group 15 herbicides results from inhibition of the VLCFA synthase, which is encoded by several fatty acid elongase (*FAEI*)-like genes in the VLCFA elongase complex in the ER (Böger 2003). The VLCFA synthase is a condensing enzyme, and relies on a conserved, reactive cysteinyl sulfur in its active site (Eckermann et al. 2003; Ghanevati and Jaworski 2002) that performs a nucleophilic attack on either the natural substrate

(fatty acyl-CoA) or herbicide (Böger et al. 2000; Götz and Böger 2004). Binding of the herbicide at the VLCFA synthase consequently limits the four-step process of elongation of long-chain fatty acids (C16 and C18) to VLCFAs through sequential incorporations of two carbons from the second substrate, malonyl-CoA (Böger 2003). The active isomer of racemic metolachlor (*S*-metolachlor) is a competitive inhibitor (with respect to acyl-CoA) of the first enzymatic step of the VLCFA elongase complex (3-ketoacyl-CoA synthase), resulting in depletion of VLCFAs (Böger 2003; Trenkamp et al. 2004). Although the MoA of Group 15 herbicides involves covalent binding, or alkylation, of the conserved cysteine residue in VLCFA condensation enzymes (Eckermann et al. 2003; Götz and Böger 2004), some Group 15 herbicides (e.g., chloroacetamides) display irreversible inhibition (alkylation), while other herbicides (e.g., pyroxasulfone and thiocarbamate-sulfoxides) show reversible inhibition (Krähmer et al. 2019; Tanetani et al. 2011b). Although the primary SoA and MoA of Group 15 herbicides are now accepted in the literature, it is important to note that many findings reported in earlier research are likely valid but may have resulted from secondary responses triggered by higher herbicide concentrations used in their experiments (reviewed by Böger et al. 2000; Götz and Böger 2004).

Different types of fatty acid elongases (FAEs) [e.g., β -ketoacyl-CoA synthase (KCS)] perform condensation reactions in plants (Ghanavati and Jaworski 2002; Haslam and Kunst 2020; Millar et al. 1999), as well as type III polyketide synthases (Eckermann et al. 2003), and thus are potential SoAs for Group 15 herbicides in weeds. Plants studied to date typically contain between 10 to 30 KCS-like genes per diploid genome (Guo et al. 2016; Huai et al. 2020). Additional biochemical research demonstrated that several Group 15 herbicides inhibit the activity of different VLCFA synthases in *Arabidopsis thaliana*, including enzymes encoded by the genes *At5g43760*, *At104220*, *At1g25450*, *KCS1*, *KCS2*, and *FAE1* (Trenkamp et al. 2004). Expression of FAEs occurs in different plant tissues and organs (Joubès et al. 2008), varies in response to stresses (Batsale et al. 2021), and is regulated at different developmental stages. Substrate specificity (i.e., which fatty acyl-CoAs are elongated) of the entire VLCFA elongase complex is determined by the first step catalyzed by FAEs (Krähmer et al. 2019; Millar and Kunst 1997; Trenkamp et al. 2004). These molecular and biochemical factors likely affect which specific FAEs are inhibited by soil-applied Group 15 herbicides, which primarily inhibit shoot growth of emerging seedlings (Fuerst 1987). Group 15 herbicides are nonionic, which precludes phloem mobility in seedlings, although limited xylem translocation from root absorption to older leaves is possible (Pillai et al. 1979). Several FAEs are expressed in the epidermal cells of shoot tissues (Batsale et al. 2021; Joubès et al. 2008), indicating that inhibition of specific target sites (and their homologs in sensitive weeds) may lead to seedling growth inhibition and death.

VLCFA Profiles in Group 15 Herbicide-Treated Plants

The VLCFA depletion in plant cell cultures and/or plant tissues following Group 15 herbicide treatment is typically measured using VLCFA precursors, including radiolabeled stearic acid (C18:0) or oleic acid (C18:1) as biomarkers for subsequent elongation steps (Böger et al. 2000; Hwang et al. 2023; Matthes et al. 1998; Tanetani et al. 2009). In the green algae *Senedesmus acutus* (Meyen), radiolabeled C18:1 was supplied to algal cultures and the levels of three VLCFAs (C22:1, C24:1, and C26:1) were measured by high-performance liquid chromatography analysis

(Böger et al. 2000). Treatment of the cultures with 1 μ M metazachlor completely inhibited the production of three VLCFAs and several more nonpolar compounds (likely VLCFAs >26C), and biochemically phenocopied the known VLCFA-deficient green algae mutant Mz-1 (Böger et al. 2000; Schmalfuß et al. 1998). In addition to severe VLCFA depletion, accumulation of C18:1 was also measured, which assisted in pinpointing the elongases as targets for inhibition by metazachlor and other Group 15 herbicides (Böger et al. 2000).

Research with cultured rice cells (Tanetani et al. 2009) or rice and other grass plants (Tanetani et al. 2011b) has been aimed at determining the SoA and MoA of pyroxasulfone. VLCFA biosynthesis was severely inhibited by pyroxasulfone at nanomolar concentrations, including specific elongation steps from C18:0 to C20:0 up to C26:0 to C28:0, as well as C18:1 to C20:1 (Tanetani et al. 2011b) with a concomitant accumulation of long-chain fatty acid precursors (e.g., C14:0 and C16:0). In preliminary research on a VLCFA inhibitor-resistant Palmer amaranth, gas chromatography was used to examine the concentration of three representative VLCFAs, namely C22:0, C24:0, and C26:0 (Hwang et al. 2023), which were derived from C18:0 substrate synthesized in plastids. This study showed that C22:0 and C24:0 levels slightly increased in *S*-metolachlor-treated seedlings in both sensitive and resistant Palmer amaranth populations, while the levels of C26:0 decreased in both populations (Hwang et al. 2023). The inhibition of specific VLCFA synthases and the depletion or accumulation of certain VLCFAs in sensitive and resistant weed populations (monocots and dicots) is an area that warrants further research to investigate possible target-site mechanisms of resistance to VLCFA-inhibiting herbicides (Busi 2014).

Use of VLCFA Inhibitor in the United States

Acetochlor and *S*-metolachlor are the most used VLCFA-inhibiting herbicides in corn and soybean crops, respectively (Figure 6). According to a survey conducted in 2021 by U.S. Department of Agriculture–National Agricultural Statistics Service, the estimated use of VLCFA-inhibiting herbicides such as acetochlor, dimethenamid-*P*, pyroxasulfone, metolachlor, and *S*-metolachlor in corn production in the United States was 18,903, 1,311, 54, 3,290, and 12, 248 metric tons, respectively (Figure 6A; USDA-NASS 2021a). The survey estimated that about 3,660, 874, 784, 2,085, and 9,116 metric tons of acetochlor, dimethenamid-*P*, pyroxasulfone, metolachlor, and *S*-metolachlor, respectively, were used on soybean crops in the United States in 2020 (Figure 6B; USDA-NASS 2020b). On average, VLCFA-inhibiting herbicides were applied about once a year to corn and soybean (USDA-NASS 2020b, 2021a).

Acetochlor

Acetochlor belongs to the α -chloroacetamides family of VLCFA-inhibitor (WSSA 2021). Acetochlor was initially labeled for PPI or PRE applications to corn to control annual monocots, some small-seeded dicots, and yellow nutsedge (*Cyperus esculentus* L.) (Shaner et al. 2014). After registration in the United States in 1994, acetochlor use increased significantly (Figure 7A; US-EPA 1994; Wieben 2019). Acetochlor was marketed with expectations that formulations with reduced rate would decrease the use of other corn herbicides such as 2,4-D, alachlor, atrazine, butylate, EPTC, and metolachlor. As a result, after 1994 the use of acetochlor in corn production fields increased gradually (Figure 7A; Capel et al.

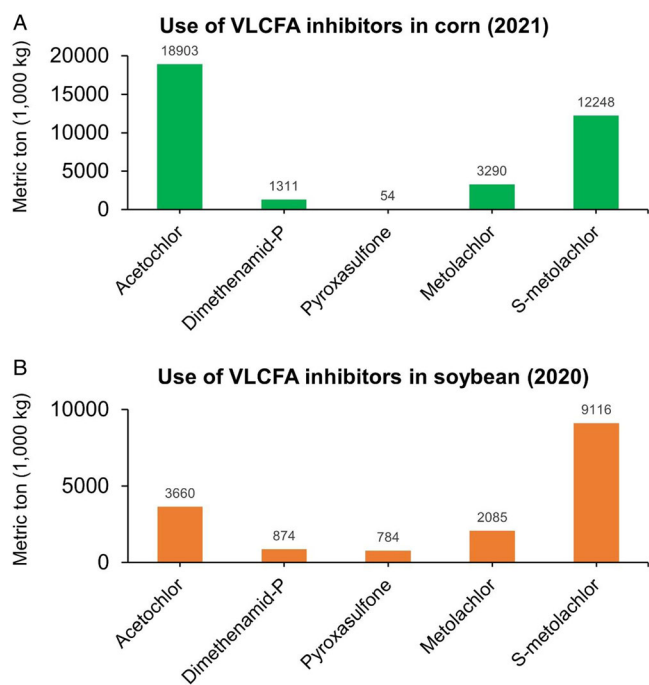


Figure 6. The use of very-long-chain fatty acid (VLCFA)-inhibiting herbicides in A) corn in 2021, and B) soybean in 2020 in the United States (USDA-NASS 2020b, 2021a).

1995; Hackett et al. 2005; Wieben 2019). During its first year of release in 1994, acetochlor was the fifth-most applied corn herbicide, and with its more extensive use in successive years, it became the third-most applied corn herbicide in the Midwestern United States in 1996 (Clark and Goolsby 1999). In 2021, acetochlor was the fourth-most applied herbicide to corn in the United States (USDA-NASS 2022). About one-third (34%) of 37.8 million ha (92.1% of the total planted area) received acetochlor (including multiple active ingredients) at an average rate of 1.59 kg ai ha⁻¹, which totaled 18,903 metric tons in 2021 (Figure 7A; USDA-NASS 2022). The major corn-producing states in the Midwestern United States used acetochlor in the range of 406 to 3,768 metric tons per state, with Iowa (3,768 metric tons), Nebraska (3,014 metric tons), Minnesota (2,878 metric tons), and South Dakota (2,312 metric tons) being the top four with the highest usage in 2021 (Figure 8A; USDA-NASS 2021a).

With the release of a micro-encapsulated formulation of acetochlor (Warrant[®]), its use has expanded in soybean, cotton, and other crops over the last decade (Figure 7A; Wieben 2019). The slow release of acetochlor from the micro-encapsulated formulation increased crop safety and extended residual weed control (Cahoon et al. 2015; Jhala et al. 2015; Parker et al. 2005; Riar et al. 2011). Acetochlor use in soybean production was comparatively less than in corn (98 to 733 metric tons per state; Figure 8). Growers in Illinois used the highest amount of acetochlor on soybean (733 metric tons) followed by Iowa (611 metric tons), Nebraska (476 metric tons), and Minnesota (458 metric tons) (Figure 8B; USDA-NASS 2020b). Almost all of Illinois and Iowa, southern Minnesota, eastern Nebraska, and South Dakota used the high-end rate of >7.57 kg km⁻² (USGS 2022; Figure 9).

S-Metolachlor

Metolachlor is a member of the α -chloroacetamides family of VLCFA-inhibiting herbicides (WSSA 2021). It is a mixture of

R- and *S*-enantiomers in a 1:1 proportion, with most of the herbicidal activity derived from *S*-isomers (Moser et al. 1983). It was widely used (>25,000 metric tons yr⁻¹ in the period 1992–1996) in the United States before the registration of *S*-metolachlor in 1997 (Figure 7B; Shaner et al. 2006; Wieben 2019). *S*-metolachlor was commercialized through an innovation in the manufacturing process when a new catalyst system enabled the selective synthesis of *S*-metolachlor at a commercial scale (Blaser and Spinder 1997). These resolved isomer formulations are listed as *S*-metolachlor and usually contain about 88% of *S*-isomers and 12% of *R*-isomers (Shaner et al. 2006). Because *S*-isomers are more biologically active than *R*-isomers, the application rate of *S*-metolachlor is 65% of the racemic mixture of metolachlor (O’Connell et al. 1998; Shaner et al. 2006). As a result, metolachlor use decreased from about 30,152 metric tons in 1996 to about 166 metric tons in 2002 (Figure 7, B, C, and D; Wieben 2019).

In 2021, *S*-metolachlor was the fifth-most-applied corn herbicide in the United States (USDA-NASS 2022). About one-fourth (27%) of 37.8 million ha of corn received *S*-metolachlor at an average rate of 1.3 kg ha⁻¹, totaling 12,248 metric tons (Figure 10A;). Illinois accounted for more than one-fourth (28%; 3,410 metric tons) of the total *S*-metolachlor applied to corn crops (Figure 10A; USDA-NASS 2021a). *S*-metolachlor was the fourth-most-applied herbicide to soybean crops in the United States in 2020 (USDA-NASS 2021b). It was applied to about one-fifth (19%) of 33.6 million ha of soybean (96.3% of the total planted area) at an average rate of 1.47 kg ha⁻¹, which accrues to 9,116 metric tons of *S*-metolachlor (Figure 10B; USDA-NASS 2020b). Illinois was the largest (15%; 1,388 metric tons) user of *S*-metolachlor followed by Iowa (942 metric tons), Minnesota (861 metric tons), and Nebraska (691 metric tons) (Figure 10B; USDA-NASS 2020b).

S-metolachlor can be applied PPI, PRE, or POST to peanut and cotton; PPI or PRE to sorghum and safflower (*Carthamus tinctorius* L.); and PRE to some turfgrass species and vegetable crops (Shaner et al. 2014). It was the third-most-applied herbicide to peanut crops in 2018, with a total of 271 metric tons of *S*-metolachlor applied to 34% of 0.57 million ha (93% of the total planted area) at an average of 1.50 kg ha⁻¹ (USDA-NASS 2019). *S*-metolachlor was the third-most-applied herbicide to sorghum in 2019, with 1,089 metric tons applied to 36% of 2.14 million ha at an average of 1.42 kg ha⁻¹ (USDA-NASS 2020a). Among vegetable crops, *S*-metolachlor was the second-most-applied herbicide on pumpkins (*Cucurbita* spp.) and snap beans (*Phaseolus vulgaris* L.) in the United States in 2020 (USDA-NASS 2021c). It was applied to 38% of pumpkin-planted area at an average rate of 1.22 kg ha⁻¹ totaling 11,748 kg, and was applied to 38% of snap bean planted area at an average of 1.31 kg ha⁻¹, totaling 37,875 kg of *S*-metolachlor (USDA-NASS 2021c).

Alachlor

Alachlor was the first widely accepted PRE herbicide from the α -chloroacetamide family and was commercialized in 1969 by Monsanto (Hamm 1974). It was applied as early preplant, PPI, PRE, or early POST to corn; PPI and/or PRE to sorghum, soybean, peanut (where it can also be applied at cracking), and lima bean (*Phaseolus lunatus* L.); PPI to dry bean; and directed-PRE to woody ornamentals (Shaner et al. 2014). It was primarily used on corn crops, followed by soybean, although its use progressively declined from the 1990s to the 2010s (Figure 11A; Wieben 2019). Wieben (2019) reported that more than 21,000 metric tons year⁻¹ of

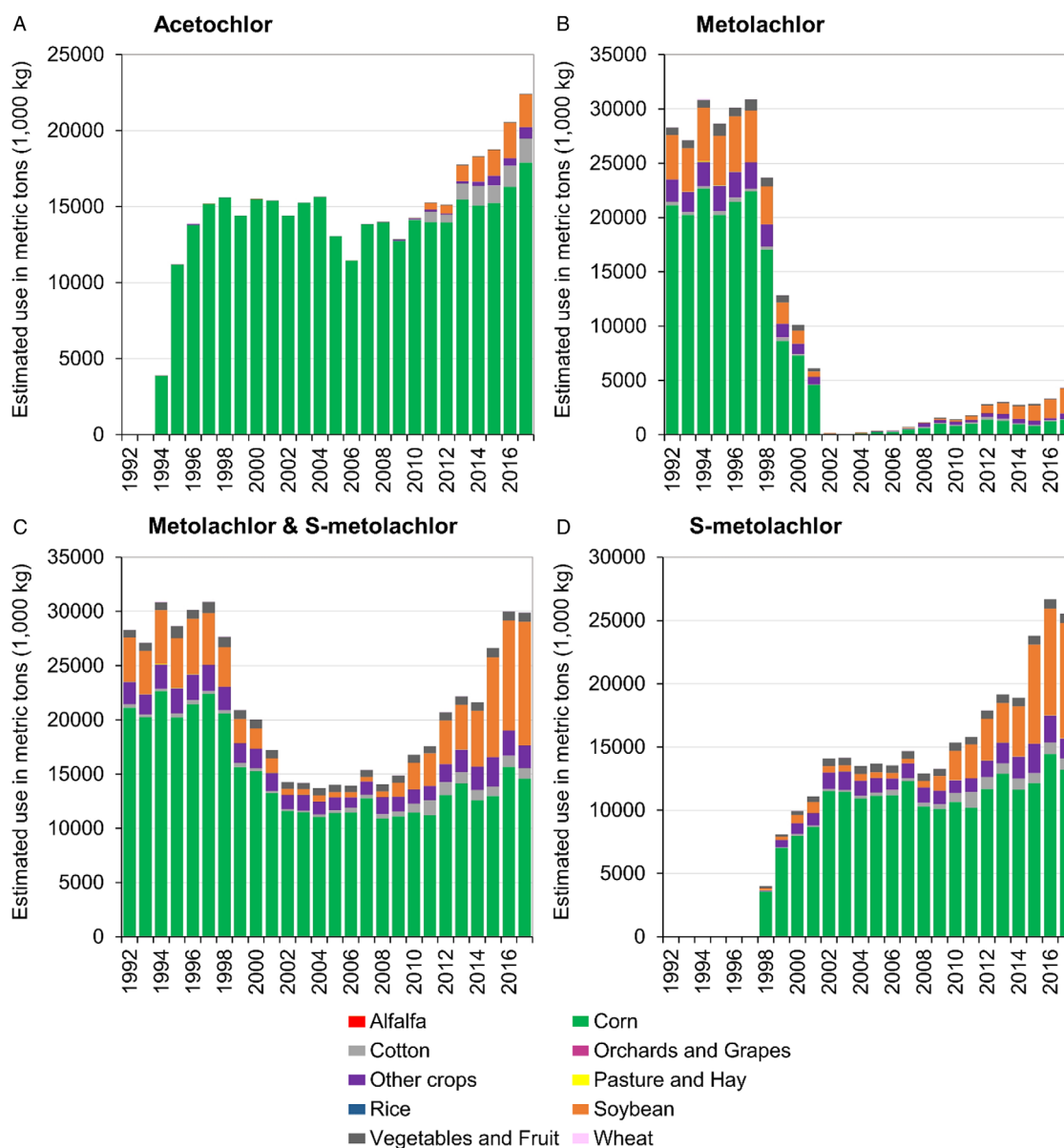


Figure 7. Estimated use of acetochlor and metolachlor in the United States from 1992 to 2017. A) Acetochlor, B) metolachlor, C) metolachlor and S-metolachlor, and D) S-metolachlor. Adapted from USGS-NAWQA (2022) with pesticide use data from Wieben (2019).

alachlor was applied during the years 1992 to 1995 compared with less than 170 metric tons year⁻¹ during the years 2015 to 2017.

Dimethenamid-*P*

Similar to metolachlor, dimethenamid-*P* is a member of the α -chloroacetamide family and has two main isomers: *R*- and *S*-isomers (Böger et al. 2000; WSSA 2021). Dimethenamid-*P* is an active *S*-isomer with greater herbicidal activity than *R*-isomer (Couderchet et al. 1997). It can be applied as early preplant, PPI, PRE, or early POST to corn and soybean (Shaner et al. 2014). According to estimates by Wieben (2019), dimethenamid-*P* use in corn was <600 metric tons yr⁻¹ in the 2000s, though its use increased progressively in the 2010s, rising by more than 5-fold in 2017 (3,390 metric tons) compared with 2007 (576 metric tons). Of the approximately 3,390 metric tons of dimethenamid-*P* used in

the United States in 2017, more than half (55%; 1,860 metric tons) was used on corn, and one-fourth (25%; 831 metric tons) on soybean (Figure 11C; Wieben 2019). In 2021, corn received slightly less (1,311 metric tons) dimethenamid-*P* (Figure 11A; USDA-NASS 2021a), whereas soybean received slightly more (874 metric tons) in 2020 (Figure 11B; USDA-NASS 2020b).

Flufenacet

Flufenacet belongs to the α -oxyacetamide family of VLCFA inhibitors (WSSA 2021). It may be used as surface preplant, PPI, and/or PRE on many crops such as corn, cotton, peanut, potato (*Solanum tuberosum* L.), soybean, sunflower (*Helianthus annuus* L.), and wheat (Shaner 2014). Flufenacet is not sold as a single active ingredient but is available as a premix for use on corn and soybean (Shaner 2014). Use of flufenacet on corn has decreased in

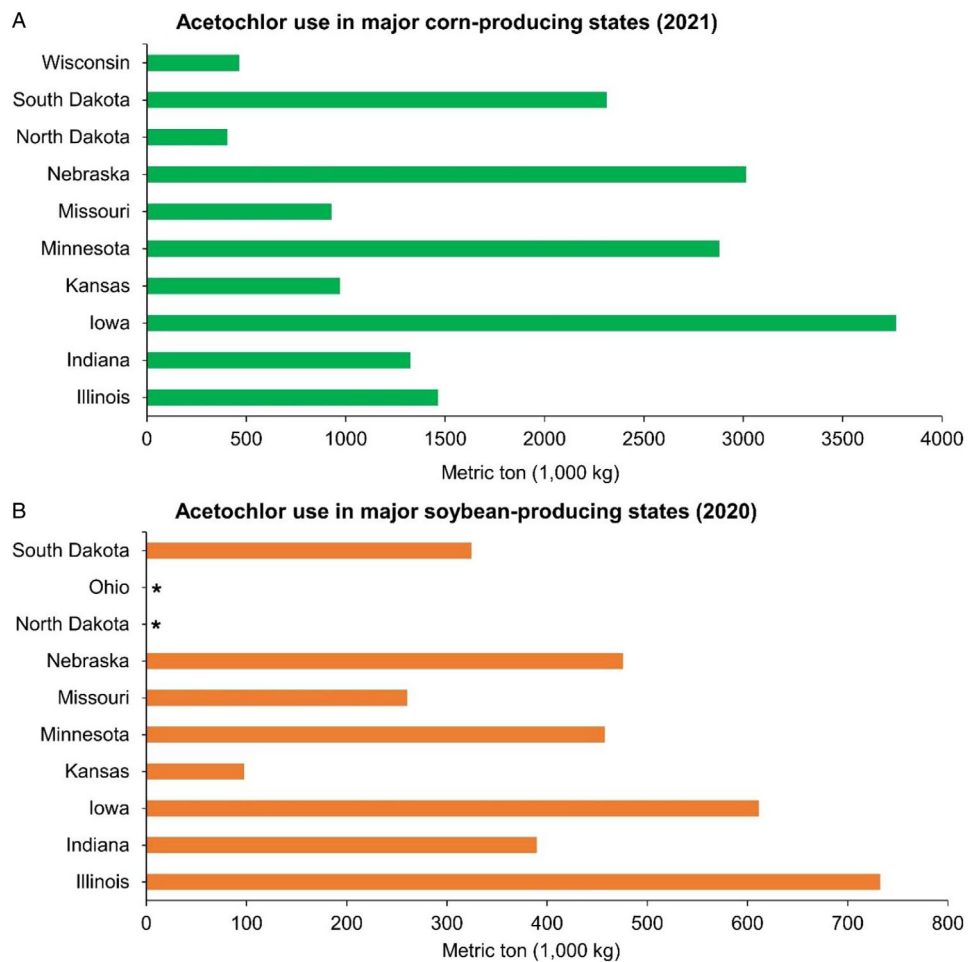


Figure 8. Acetochlor used in the major A) corn-producing and B) soybean-producing states of the United States. An asterisk (*) indicates that data were not disclosed for those states (USDA-NASS 2020b; 2021a).

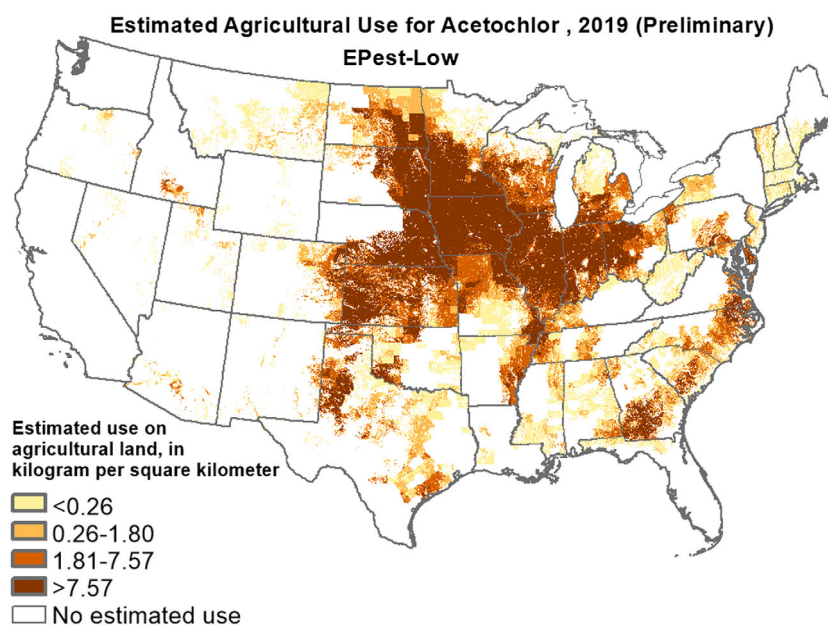


Figure 9. Acetochlor usage on agricultural land across the United States in 2019 (downloaded and modified from the U.S. Geological Survey by the U.S. Department of the Interior) (USGS 2022).

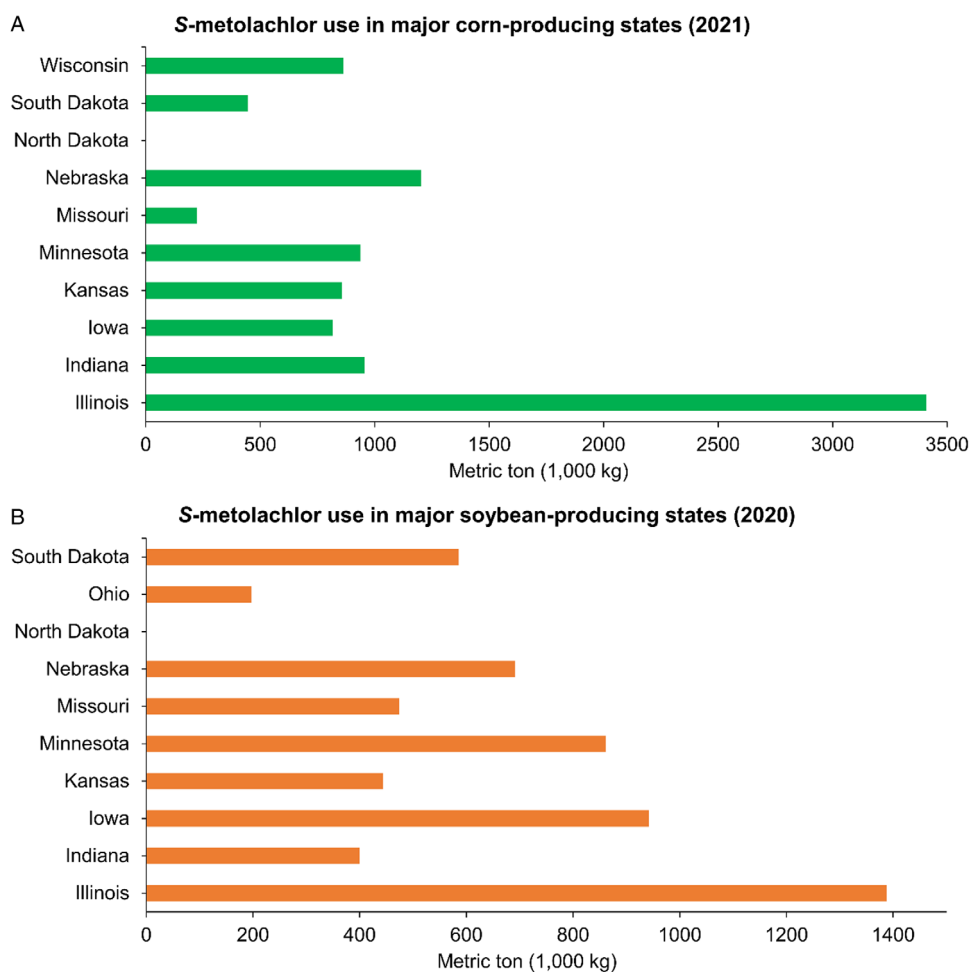


Figure 10. S-metolachlor used in the major A) corn-producing and B) soybean-producing states of the United States. An asterisk (*) indicates that data were not disclosed for North Dakota (USDA-NASS 2020b, 2021a).

the last decade (<40 metric tons yr^{-1} from 2011 to 2015) compared with the 2000s (>250 metric tons yr^{-1} from 2001 to 2005) (Figure 11B; Wieben 2019).

Propachlor and Pyroxasulfone

Among VLCFA-inhibiting herbicides, propachlor was commercialized along with CDAA (N,N-diallyl-2-chloroacetamide) by Monsanto in 1965 (Table 1; Heydens et al. 2010; Shaner et al. 2014). The granular formulation of propachlor used to cause skin irritation (eye and nose), though less than CDAA (Hamm 1974; Pike et al. 1991), leading propachlor to replace CDAA. However, alachlor took over the market after the 1970s, as it was less irritating than propachlor while offering a similar level of weed control (Pike et al. 1991). Propachlor was applied PRE to corn and sorghum (Shaner 2014), and based on propachlor usage data from 1987 to 1996, an average of about 959 metric tons of propachlor was used annually, with 75% applied to sorghum, and 24% to corn (US-EPA 1998). In 1998, Monsanto voluntarily discontinued production of propachlor (Gómez-Ramírez and García-Fernández 2014) and its use has since decreased (Figure 11E; Wieben 2019).

Pyroxasulfone belongs to the isoxazoline family (WSSA 2021). Among VLCFA inhibitors, it is the most recently commercialized PRE herbicide, registered by Kumiai Chemical Industry Co., Ltd in 2014 (Table 1). Pyroxasulfone can be applied from autumn, early

preplant, to early POST during the growing season and provides residual control of troublesome weeds that are resistant to ACCase inhibitors, ALS inhibitors, glyphosate, and triazines (Grey et al. 2013; Kaur et al. 2019; Shaner et al. 2014; Umetsu and Shirai 2020). Pyroxasulfone has a relatively lower use rate compared to chloroacetamides (Zollinger 2011). It provides longer residual activity against troublesome broadleaf weeds such as Palmer amaranth and waterhemp (Nakatani et al. 2016). In 2013, approximately 65 metric tons of pyroxasulfone was applied in the United States, and subsequently >250 metric tons yr^{-1} from 2014 to 2017 (Figure 6; Wieben 2019). In 2021, about 54 metric tons of pyroxasulfone were applied to corn (Figure 11F; USDA-NASS 2021a), whereas in 2020, 784 metric tons were applied to soybean (Figure 11F; USDA-NASS 2020b).

VLCFA-Inhibiting Herbicide-Resistant Weeds

The evolution of weeds that are resistant to VLCFA inhibitors is relatively low compared to ALS or Photosystem II (PS II) inhibitors (Heap 2023). Several factors might have contributed to the low incidence of weeds that are resistant to VLCFA inhibitors, including possible low frequency of mutations in condensing enzymes in the long-chain fatty acid synthesis (Tanetani et al. 2009; Trenkamp et al. 2004), genetic redundancy of fatty acid elongases (Brabham et al. 2019), and the predominant use of these herbicides

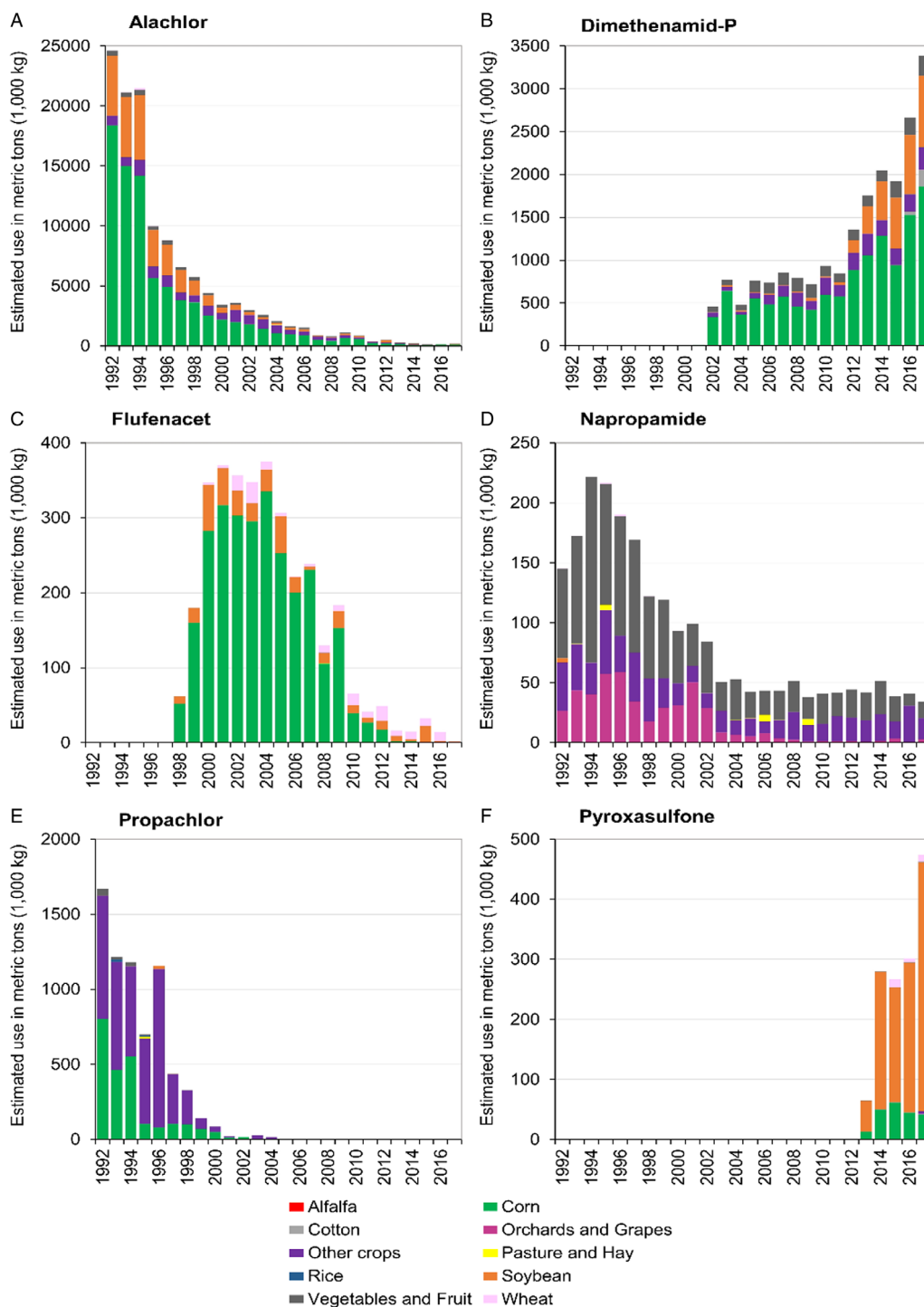


Figure 11. Estimated use of very-long-chain fatty acid (VLCFA)-inhibiting herbicides in the United States from 1992 to 2018. A) Alachlor, B) flufenacet, C) dimethenamid-P, D) napropamide, E) propachlor, and F) pyroxasulfone. Adapted from USGS-NAWQA (2022), with pesticide use data from Wieben (2019). Napropamide belonged to the acetamide family of Group 15 herbicides in the previous herbicide classification; however, in the revised classification, it is classified as a Group 0 herbicide (WSSA 2021).

for PRE application followed by a POST application of other herbicides, making it difficult for surviving weeds to grow to maturity and produce seeds (Busi et al. 2014). As of November 2023, resistance to the VLCFA inhibitor has been documented in 13 weed species (Heap 2023; Table 3). The VLCFA inhibitor resistance has been found in 11 monocot weeds and two dicot weeds, Palmer amaranth and waterhemp (Heap 2023; Table 3), the two most problematic weeds of many cropping systems in the United States (WSSA 2017).

Mechanism of Resistance to VLCFA-Inhibiting Herbicides in Weed Species

Blackgrass

Resistance to multiple herbicides is common in several blackgrass (*Alopecurus myosuroides* Huds.) populations throughout Europe. The first case of a flufenacet-resistant blackgrass was reported in 2007 in Germany and later in 2011 in Sweden (Heap 2023). The enhanced activity of GSTs was attributed to resistance in several

Table 3. Weeds that are resistant to very-long-chain fatty acid-inhibiting herbicides worldwide.

Weed species	Country reported	Resistance to	Reference
Rigid ryegrass (<i>Lolium rigidum</i>)	Australia	Metolachlor, Triallate, Pyroxasulfone, Prosulfocarb	Busi et al. 2012; Busi and Powles 2013; Brunton et al. 2018; 2019
Annual ryegrass (<i>Lolium perenne</i> spp. <i>multiflorum</i>)	France, Australia, U.K., USA	Flufenacet	Suzukawa et al. 2021; Liu et al. 2016; Rauch et al. 2010, Dücker et al. 2019a
Giant parammatta grass (<i>Sporobolus fertilis</i>)	Australia	Flupropanate, Dalapon	Ramasamy et al. 2008a
Serrated tussock grass (<i>Nassella trichotoma</i>)	Australia	Flupropanate	Ramasamy et al. 2008b
Chilean needlegrass (<i>Nassella neesina</i>)	New Zealand	Dalapon	Hartley 1994
Wild oat (<i>Avena Fatua</i>)	Canada USA	Triallate, Pyroxasulfone Triallate	Beckie et al. 2012; Mangin et al. 2016 Heap 2023
Barnyardgrass (<i>Echinochloa crus-galli</i> var. <i>crus-galli</i>)	China	Thiobencarb, Butachlor	Chen et al. 2016
	Philippines	Butachlor	Juliano et al. 2010
	Thailand	Butachlor	Heap 2023
	USA	Thiobencarb, Molinate	Fischer et al. 2000
Early watergrass (<i>Echinochloa oryzoides</i>)	USA	Thiobencarb, Molinate	Fischer et al. 2000
Late watergrass (<i>Echinochloa phyllopogon</i>)	USA	Thiobencarb, Molinate	Fischer et al. 2000
Blackgrass (<i>Alopecurus Myosuroides</i>)	Germany Sweden France U.K.	Flufenacet Prosulfocarb Pyroxysulfone Flufenacet	Dücker et al. 2019b Keshtkar et al. 2015 Dücker et. 2019b; 2020
Palmer amaranth (<i>Amaranthus palmeri</i>)	USA	S-metolachlor	Brabham et al. 2019
Waterhemp (<i>Amaranthus tuberculatus</i>)	USA	Acetochlor, Alachlor, S- metolachlor, Pyroxasulfone	Evans et al. 2019; Strom et al. 2020
Annual bluegrass (<i>Poa annua</i>)	USA	Ethofumesate	Heap 2023

blackgrass populations (Dücker et al. 2020). The use of GST inhibitor (e.g., tridiphane) slowed degradation of flufenacet in sensitive and resistant blackgrass populations (Dücker et al. 2019b). The RNA sequencing indicated an increased expression of six GSTs and nine transcription factors, as well as a keto-acyl-CoA reductase, which is known to be involved in the biosynthesis of VLCFA inhibitor (Dücker et al. 2020).

Echinochloa Species

Barnyardgrass [*Echinochloa Crus-galli* (L.) Beauv] is a dominant weed in direct-seeded rice (Chauhan et al. 2011), and early watergrass [*Echinochloa oryzoides* (Ard.) Fritsch] and late watergrass (*Echinochloa phyllopogon*) are commonly found in lowland rice cropping systems (Fischer et al. 2000). Butachlor and triallate are widely used in rice fields for weed management, including for control of *Echinochloa* spp. Butachlor-resistant barnyardgrass was first reported in China in 1993 (Heap 2023), and now several populations in Asia are resistant to this herbicide (Juliano et al. 2010). Early watergrass and late watergrass populations with 5-fold to 22-fold resistance to molinate and thiobencarb were reported (Fischer et al. 2000), and these populations also exhibit resistance to inhibitors of ACCase, ALS, and PS II as well as synthetic auxins (Fischer et al. 2000; Busi 2014). Physiological and biochemical analyses have revealed indirect evidence of metabolism of thiobencarb via cytochrome P450 (CYP) activity in late watergrass (Yun et al. 2005). Studies involving transgenic *Arabidopsis thaliana* expressing the *CYP81A12/21* gene indicated that CYP provides cross-resistance to ALS and ACCase inhibitors, but not to thiocarbamate (thiobencarb) (Dimaano et al. 2022), suggesting that resistance to thiobencarb may involve different cluster of CYPs (Dimaano et al. 2022).

Palmer Amaranth

Resistance to VLCFA-inhibiting herbicides in a Palmer amaranth biotype was first reported in Arkansas, where a 3-fold to 29-fold resistance to S-metolachlor was found (Rangani et al. 2021). Additional accessions of Palmer amaranth from Arkansas and Mississippi were found to have 2-fold to 7-fold resistance to S-metolachlor, while the progenies generated from resistant plants were 9.2 times less sensitive than the susceptible population (Kouame et al. 2022). In another Palmer amaranth population from Arkansas, an 8-fold resistance to S-metolachlor was reported, with a low level (2.3-fold to 3.6-fold) of cross-resistance to other Group 15 herbicides (Brabham et al. 2019).

Treatment with a known GST inhibitor (NBD-Cl) indirectly implied the involvement of GSTs in metabolizing S-metolachlor in Palmer amaranth plants (Rangani et al. 2021). That research (Rangani et al. 2021) suggested that the inactivation of herbicide by enhanced activity of GSTs was predominant in roots compared with leaves. Additionally, two GST genes, *ApGSTU19* and *ApGSTF8*, were constitutively upregulated in the roots of resistant plants, and the expression of these genes increased in response to S-metolachlor. Gene expression analyses revealed the upregulation of GST gene clusters *ApGSTU19*, *ApGSTF8*, *ApGSTF2*, and *ApGSTF2like*, which appear to be responsible for increased GST activity in resistant plants (Rangani et al. 2021). Thus, it is likely that the resistance to S-metolachlor in Palmer amaranth is bestowed because of elevated constitutive and induced expression of GST genes (Rangani et al. 2021). Similarly, in another Palmer amaranth population from Arkansas, using NBD-Cl (4-chloro-7-nitrobenzofurazan), Brabham et al. (2019) suggested the possible role of GST in S-metolachlor metabolism and, thereby resistance.

Although the studies described above provide reason to believe that GSTs are indirectly involved in metabolizing S-metolachlor in

Palmer amaranth, recent studies suggest that CYPs contribute to *S*-metolachlor resistance in Palmer amaranth (Concepcion et al. 2023). Rapid metabolism of herbicidally active *S*-metolachlor to polar compounds (including putative glutathione, dipeptide, and cysteine conjugates) was noted to have occurred, but some metabolites did not match the migration distances of the glutathione-derived conjugates, implying that some *S*-metolachlor metabolites may arise from Phase I oxidation and Phase II glucose conjugation via CYP activity in resistant Palmer amaranth seedlings (Concepcion et al. 2023). Those results suggest that CYPs are equally involved in *S*-metolachlor metabolism as GSTs.

Rigid Ryegrass

Resistance to triallate and metolachlor was reported in a rigid ryegrass (*Lolium rigidum* Gaud.) population from Australia (Heap 2023). Resistance to pyroxasulfone and other chloroacetamides was found later in rigid ryegrass (Burnet et al. 1994; Busi et al. 2012, 2014). The pyroxasulfone-resistant population was also cross-resistant to prosulfocarb and triallate (Busi and Powles 2013, 2016). The rigid ryegrass population exhibited 6-fold to 31-fold resistance to *S*-metolachlor and was cross-resistant to alachlor and propachlor (Burnet et al. 1994; Brunton et al. 2019). Importantly, resistance levels of 17- to 44-fold, 6- to 45-fold, and 4- to 8-fold to triallate, prosulfocarb, and pyroxasulfone, respectively, were reported in this species (Brunton et al. 2019, 2020). Rigid ryegrass biotypes with greater than 9-fold resistance to triallate, prosulfocarb, ETPC, thiobencarb, and trifluralin have also been found (Brunton et al. 2018).

Rapid metabolism of chloroacetamides (*S*-metolachlor) (Burnet et al. 1994) and pyroxasulfone (Tanetani et al. 2013) possibly mediated by CYP activity may confer resistance in rigid ryegrass (Burnet et al. 1994; Tanetani et al. 2013); however, direct evidence of CYPs in metabolizing these herbicides is elusive in this weed species (Busi 2014). Nonetheless, in another pyroxasulfone-resistant rigid ryegrass population, rapid metabolism of [¹⁴C] pyroxasulfone (>88%) via a glutathione conjugation mediated by GST was found (Busi et al. 2018). Additionally, pyroxasulfone-resistant rigid ryegrass showed a significant increase in constitutive expression of *tau* classes of GST genes (*GST-1* and *GST-2*) compared with susceptible plants (Busi et al. 2018). Similarly, conjugation of flufenacet via GST activity was found in a rigid ryegrass population from Europe (Dücker et al. 2019a). The use of phorate showed synergism with enhanced activity of pyroxasulfone with reduced resistance (Busi and Powles 2016). Conversely, phorate antagonized thiocarbamate herbicides (prosulfocarb and triallate) with an increased activity of thiocarbamates via bioactivation (Fuerst 1987). Busi et al. (2014) demonstrated that the resistance to pyroxasulfone, prosulfocarb, and triallate is conferred by one or more semidominant alleles.

Waterhemp

Two populations of waterhemp from Illinois were reported to be resistant to VLCFA-inhibiting herbicides, including *S*-metolachlor (Evans et al. 2019; Strom et al. 2019). These populations were also resistant to ALS-, HPPD-, and PS II-inhibitor (Heap 2023). The resistant plants degraded *S*-metolachlor rapidly than sensitive plants, and similar to corn at 2–24 h after treatment (HAT) (Strom et al. 2020). The use of GST and CYP-inhibitor decreased the amount of *S*-metolachlor metabolized by the resistant plants at 4 HAT but not in sensitive waterhemp or naturally tolerant corn plants (Strom et al. 2019). Interestingly, thin-layer chromatography (TLC) analysis of radiolabeled *S*-metolachlor indicated that

resistant plants formed metabolites that were not present in sensitive waterhemp or corn (Strom et al. 2019). It was revealed that conjugates of *O*-demethylated *S*-metolachlor were abundant in resistant plants compared to susceptible waterhemp plants. The resistant waterhemp had a greater ability to oxidize *S*-metolachlor through *O*-demethylation than susceptible waterhemp or corn, and this oxidation reaction appears to be the predominant resistance mechanism in waterhemp (Storm et al. 2021). Importantly, the microsomal stability assays indicate greater than 20-fold activity of CYPs in resistant waterhemp compared to GST (~2-fold) implying Phase I and Phase II metabolism of *S*-metolachlor, likely mediated by CYPs (Strom et al. 2021). Thus, results indicate that *S*-metolachlor-resistant waterhemp possibly uses a different or additional pathway to detoxify *S*-metolachlor than corn (Strom et al. 2019; 2021).

Wild Oat

The first case of triallate resistance was reported in wild oat (*Avena fatua* L.) in 1989 in Canada (Heap 2023). The triallate-resistant wild oat exhibits cross-resistance to difenzoquat (O'Donovan et al. 1994). While the precise mechanism of resistance was unknown, Rashid et al. (1998) proposed that elevated levels of endogenous gibberellins may contribute to the resistance. Beckie and Jana (2000) reported a case of resistance to triallate in wild oat in a field with a 10- to 15-yr history of triallate use. Interestingly, triallate resistance is often associated with resistance to pyroxasulfone and sulfentrazone without use history (Mangin et al. 2016). Wild oat populations that are resistant to these herbicides were found in several states in the United States in the 1990s (Heap 2023). Some populations exhibited differential metabolism of triallate (Kern et al. 1996), while others had reduced sulfoxidation (i.e., the conversion of triallate to the active triallate sulfoxide) (Kern et al. 1997). Kern et al. (2002) demonstrated that two recessive genes endow triallate resistance in wild oat. Such inheritance can delay the incidence of resistance, as the recessive traits tend to spread slower than the dominant or semidominant traits.

Although resistance to VLCFA inhibitors in weeds is slow to evolve, more cases of weed species becoming resistant to this group of herbicides have been recently documented (Heap 2023). The predominance of GST-mediated metabolism of these herbicides was present in the resistant populations, which were found to be resistant to other herbicides such as inhibitors of ACCase, ALS, HPPD, and PS II. Best management practices should be adopted to protect this chemistry for sustainable weed management because of their widespread use in row, vegetable, and fruit crops.

The Future of VLCFA-Inhibiting Herbicides

The isoxazolines, including pyroxasulfone and fenoxasulfone, discovered by Kumiai Chemical Industry Inc., were the latest subclass of VLCFA-inhibiting herbicides commercialized in 2014 (Table 1). Pyroxasulfone has experienced substantial growth in the marketplace for use on cereal grains, soybeans, corn, cotton, and some vegetable crops, whereas fenoxasulfone is labeled for control of *Echinochloa* spp. and other annual weeds in rice crops (Fujinami et al. 2019). The favorable environmental attributes of isoxazolines as low-use-rate VLCFA-inhibiting herbicides with relatively fewer cases of resistant weeds has prompted discovery research efforts in the last two decades. Related pyrimidinone-substituted isoxazolines (Figure 12A) were investigated by DuPont Chemicals Company, with field trials on corn, soybean, and cereals (Smith et al. 2009). Researchers at the Chinese Academy of Agricultural

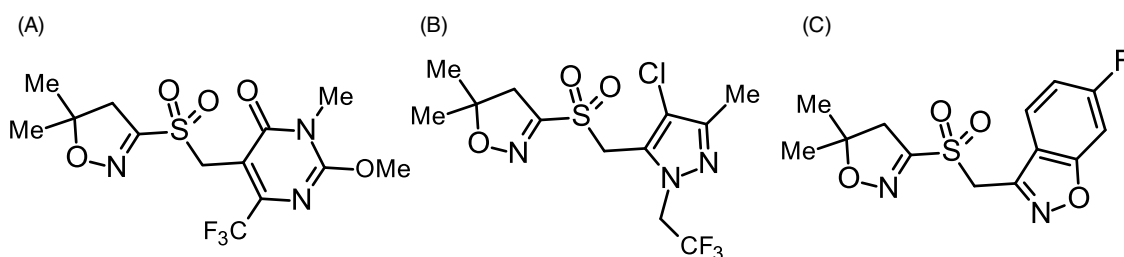


Figure 12. (A) Pyrimidinone-substituted isoxazolines investigated by DuPont Chemicals Company, (B) potent *N*-trifluoroethylpyrazole-substituted isoxazolines with encouraging herbicidal activity and corn selectivity at the Chinese Academy of Agricultural Sciences, and (C) a family of benzisoxazole-substituted isoxazolines at Dongguan HEC Pesticide R&D Company, China.

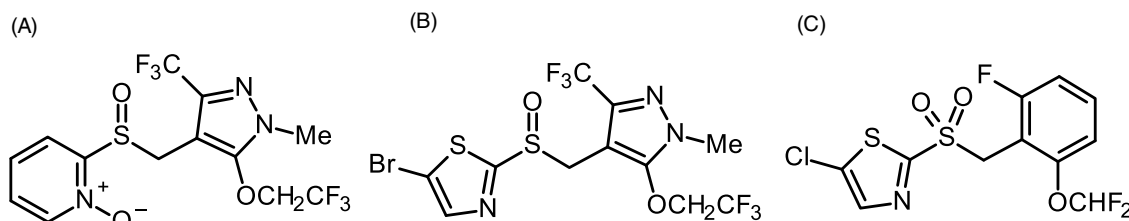


Figure 13. Different bioisosteres of the isoxazolines (A) pyridine-*N*-oxide substituted sulfoxides discovered by DuPont Chemicals Company, (B) thiazole substitutes sulfoxides discovered by Syngenta Corporation, and (C) benzylsulfonylethiazoles discovered by OAT Agrio (Japan).

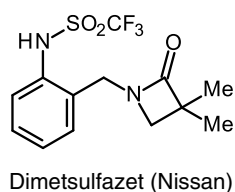


Figure 14. Dimesulfazet was discovered by Nissan Chemical Industries based on symptomatology and endogenous fatty acid analysis (Takamasa et al. 2023).

Sciences reported a series of potent *N*-trifluoroethylpyrazole-substituted isoxazolines that had encouraging weed control activity along with corn selectivity in the greenhouse and field studies at rates lower than that of metolachlor (Ma et al. 2010) (Figure 12B). Researchers at Dongguan HEC Pesticides R&D Company in China published a family of benzisoxazole-substituted isoxazolines (Figure 12C) that was competitive with pyroxasulfone to control some weed species, with improved crop safety in rapeseed, rice, and wheat crops (Lin et al. 2021). Substantial research efforts have been extended in search of bioisosteres of isoxazolines. Replacement of the isoxazoline ring by a pyridine-*N*-oxide to compounds such as sulfoxide (Figure 13A) was disclosed in a patent application by DuPont Chemicals as a highly active herbicide (Selby et al. 2008). Syngenta Corporation has disclosed sulfoxides (Figure 13B) in which thiazole replaced the isoxazoline ring (Elliott et al. 2006). A published world patent application from OAT Agrio Company (Japan) in 2018 disclosed benzylsulfonylethiazoles (Figure 13C) in which thiazole replaced the isoxazoline ring for controlling barnyardgrass, *Monochoria vaginalis*, and *Scirpus juncoides* at 200 g ai ha⁻¹ in paddy rice (Fukunaga et al. 2018).

A new direction in VLCFA-inhibiting herbicide research has been undertaken with a renewed interest in haloalkylsulfonanilide herbicides. Although they were discovered decades ago, the haloalkylsulfonanilides mefluidide and perfluidone, had an unknown SoA, though evidence was ultimately reported in 2012 to support the KAS (3-ketoacyl-ACP synthase) enzyme involved in

VLCFA biosynthesis (Tresch et al. 2012). In conjunction with this finding, Nissan Chemical Industries began to explore an alternative haloalkylsulfonanilide chemotype, starting with a patent in 2010 (Kudou et al. 2010). Nissan's continued work in this area eventually led to the commercial development of rice herbicide dimesulfazet, an inhibitor of VLCFA biosynthesis. Recently, Nissan introduced dimesulfazet based on symptomatology and endogenous fatty acid analysis (Takamasa et al. 2023; Figure 14). Dimesulfazet is being developed to control weeds of rice in Japan such as *Echinochloa oryzicola*, *Schoenoplectiella juncoides* (Roxb) Lye, and other difficult-to-control perennial species of the Cyperaceae family such as kuro-guwai (*Eleocharis kuroguwai* Ohwi) and sea clubrush [*Bolboschoenus maritimus* (L.) Palla] (Takamasa et al. 2023; Uetsu and Shirai 2020). Although the intensity of VLCFA-directed research efforts has decreased somewhat over the past decade as evidenced by the declining number of patent applications, this biochemical pathway remains a viable mechanistic target for herbicide discovery, especially in the search for new Group 15 chemotypes.

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References

- Abulnaja KO, Tighe CR, Harwood JL (1992) Inhibition of fatty acid elongation provides a basis for the action of the herbicide, ethofumesate, on surface wax formation. *Phytochem* 31:1155–1159
- Albuquerque NCP, Carrão DB, Habenschus MD, Oliveira ARM (2018) Metabolism studies of chiral pesticides: A critical review. *J Pharm Biomed Anal* 147:89–109
- Bach L, Faure JD (2010) Role of very-long-chain fatty acids in plant development, when chain length does matter. *C R Biol* 333:361–370
- Batsale M, Bahammou D, Fouillen L, Mongrand S, Joubès J, Domergue F (2021) Biosynthesis and functions of very-long-chain fatty acids in the responses of plants to abiotic and biotic stresses. *Cells* 10:1284

- Beckie HJ, Jana S (2000) Selecting for triallate resistance in wild oat. *Can J Plant Sci* 80:665–667
- Beckie HJ, Warwick SI, Sauder CA (2012) Basis for herbicide resistance in Canadian populations of wild oat (*Avena fatua*). *Weed Sci* 60:10–18
- Blaser HU, Spindler F (1997) Enantioselective catalysis for agrochemicals: the case history of the DUAL MAGNUM® herbicide. *Chimia* 51:297–299
- Böger P (2003) Mode of action for chloroacetamides and functionally related compounds. *J Pestic Sci* 28:324–329
- Böger P, Matthes B, Schmalfuß J (2000) Towards the primary target of chloroacetamides—new findings pave the way. *Pest Manag Sci* 56:497–508
- Brabham C, Norsworthy JK, Houston MM, Varanasi VK, Barber T (2019) Confirmation of S-metolachlor resistance in Palmer amaranth (*Amaranthus palmeri*). *Weed Technol* 33:720–726
- Brunton DJ, Boutsalis P, Gill G, Preston C (2018) Resistance to multiple PRE herbicides in a field-evolved rigid ryegrass (*Lolium rigidum*) population. *Weed Sci* 66:581–585
- Brunton DJ, Boutsalis P, Gill G, Preston C (2019) Resistance to very-long-chain fatty-acid (VLCFA)-inhibiting herbicides in multiple field-selected rigid ryegrass (*Lolium rigidum*) populations. *Weed Sci* 67:267–272
- Brunton DJ, Boutsalis P, Gill G, Preston C (2020) Control of thiocarbamate-resistant rigid ryegrass (*Lolium rigidum*) in wheat in southern Australia. *Weed Technol* 34:19–24
- Burnet MW, Barr AR, Powles SB (1994) Chloroacetamide resistance in rigid ryegrass (*Lolium rigidum*). *Weed Sci* 42:153–157
- Busi R (2014) Resistance to herbicides inhibiting the biosynthesis of very-long-chain fatty acids. *Pest Manag Sci* 70:1378–1384
- Busi R, Gaines TA, Vila-Aiub MM, Powles SB (2014) Inheritance of evolved resistance to a novel herbicide (pyroxasulfone). *Plant Sci* 217:127–134
- Busi R, Gaines TA, Walsh MJ, Powles SB (2012) Understanding the potential for resistance evolution to the new herbicide pyroxasulfone: field selection at high doses versus recurrent selection at low doses. *Weed Res* 52:489–499
- Busi R, Porri A, Gaines TA, Powles SB (2018) Pyroxasulfone resistance in *Lolium rigidum* is metabolism-based. *Pestic Biochem Physiol* 148:74–80
- Busi R, Powles SB (2016) Cross-resistance to prosulfocarb + S-metolachlor and pyroxasulfone selected by either herbicide in *Lolium rigidum*. *Pest Manag Sci* 72:1664–1672
- Busi R, Powles SB (2013) Cross-resistance to prosulfocarb and triallate in pyroxasulfone-resistant *Lolium rigidum*. *Pest Manag Sci* 69:1379–1384
- Cahoon CW, York AC, Jordan DL, Everman WJ, Seagroves RW, Braswell LR, Jennings KM (2015) Weed control in cotton by combinations of microencapsulated acetochlor and various residual herbicides applied preemergence. *Weed Technol* 29:740–750
- Capel PD, Ma L, Schroyer BR, Larson SJ, Gilchrist TA (1995) Analysis and detection of the new corn herbicide acetochlor in river water and rain. *Environ Sci Technol* 29:1702–1705
- Chauhan BS, Singh VP, Kumar A, Johnson DE (2011) Relations of rice seeding rates to crop and weed growth in aerobic rice. *Field Crops Res* 121:105–115
- Chen G, Wang Q, Yao Z, Zhu L, Dong L (2016) Penoxsulam-resistant barnyardgrass (*Echinochloa crus-galli*) in rice fields in China. *Weed Biol Manag* 16:16–23
- Clark GM, Goolsby DA (1999) Occurrence and transport of acetochlor in streams of the Mississippi River basin. *J Environ Qual* 28:1787–1795
- Concepcion JCT, Brown NK, Morris JA, Hutchings S, Kaundun SS, Riechers DE (2023) Comparative analysis of S-metolachlor metabolism among Palmer amaranth (*Amaranthus palmeri*) and waterhemp (*A. tuberculatus*) populations from the U.S. Abstract 397 in Proceedings of the Weed Science Society of America Annual Meeting. Arlington, Virginia, January 30–February 2, 2023
- Couderchet M, Bocion PF, Chollet R, Seckinger K, Böger P (1997) Biological activity of two stereoisomers of the N-thienyl chloroacetamide herbicide dimethenamid. *Pestic Sci* 50:221–227
- Dimaano N G, Tominaga T, Iwakami S (2022) Thiobencarb resistance mechanism is distinct from CYP81A-based cross-resistance in late watergrass (*Echinochloa phyllopogon*). *Weed Sci* 70:160–166
- Dücker R, Parcharidou E, Beffa R (2020) Flufenacet activity is affected by GST inhibitors in blackgrass (*Alopecurus myosuroides*) populations with reduced flufenacet sensitivity and higher expression levels of GSTs. *Weed Sci* 68:451–459
- Dücker R, Zöllner P, Lümmen P, Ries S, Collavo, A, Beffa R (2019a) Glutathione transferase plays a major role in flufenacet resistance of ryegrass (*Lolium spp.*) field populations. *Pest Manag Sci* 75:3084–3092
- Dücker R, Zöllner P, Parcharidou E, Ries S, Lorentz L, Beffa R (2019b) Enhanced metabolism causes reduced flufenacet sensitivity in blackgrass (*Alopecurus myosuroides* Huds.) field populations. *Pest Manag Sci* 75:2996–3004
- Eckermann C, Matthes B, Nimtz M, Reiser V, Lederer B, Böger P (2003) Covalent binding of chloroacetamide herbicides to the active site cysteine of plant type III polyketide synthases. *Phytochemistry* 64:1045–1054
- Elliott AC, Hughes P, Plant A, inventors; Syngenta Limited, assignee (2006) 4-(thiazol-2-ylthioalkyl)-pyrazoles and their use as herbicides. World Patent WO2006123088. 2006 Nov 23
- Evans CM, Strom SA, Riechers DE, Davis AS, Tranel PJ, Hager AG (2019) Characterization of a waterhemp (*Amaranthus tuberculatus*) population from Illinois resistant to herbicides from five site-of-action groups. *Weed Technol* 33:400–410
- Fedtko C (1991) Mode of action studies with mefenacet. *Pestic Sci* 33:421–426
- Fischer AJ, Ateh CM, Bayer DE, Hill JE (2000) Herbicide-resistant *Echinochloa oryzoides* and *E. phyllopogon* in California *Oryza sativa* fields. *Weed Sci* 48:225–230
- Fuerst EP (1987) Understanding the mode of action of the chloroacetamide and thiocarbamate herbicides. *Weed Technol* 1:270–277
- Fujinami M, Takahashi Y, Tanetani Y, Ito M, Nasu M (2019) Development of a rice herbicide, fenoxasulfone. *J Pestic Sci* 44:282–289
- Fukunaga S, Sumitomo T, Sumiyoshi H, Noyama S, Shirai Y, Norimura Y, Matsuzaki K, inventors; OAT Agrico Co Ltd, assignee (2018) Thiazole compounds and Herbicides. World Patent WO2018155661. 2018 Aug 30
- Ghanevati M, Jaworski J (2002) Engineering and mechanistic studies of the *Arabidopsis* FAE1 β -ketoacyl-CoA synthase, FAE1 KCS. *Eur J Biochem* 269:3531–3539
- Gómez-Ramírez P, García-Fernández AJ (2014) Propachlor. Pages 1028–1085 in Wexler P, ed. *Encyclopedia of Toxicology*, 3rd ed. Amsterdam: Elsevier–Academic Press
- Götz T, Böger P (2004) The very-long-chain fatty acid synthesis is inhibited by chloroacetamides. *Z Naturforsch* 59:549–553
- Grey TL, Cutts GS III, Newsome LJ, Newell SH III (2013) Comparison of pyroxasulfone to soil residual herbicides for glyphosate resistant Palmer amaranth control in glyphosate resistant soybean. *Crop Manag* 12:1–6
- Guo H-S, Zhang, Y-M, Sun X-Q, Li M-M, Hang YY, Xu J-Y (2016) Evolution of the KCS gene family in plants: the history of gene duplication, sub/neofunctionalization and redundancy. *Mol Genet Genom* 291:739–752
- Hackett AG, Gustafson DI, Moran SJ, Hendley P, Van Wesenbeck I, Simmons ND, Klein AJ, Kronenberg JM, Fuhrman JD, Honegger JL, Hanzas J (2005) The acetochlor registration partnership surface water monitoring program for four corn herbicides. *J Environ Qual* 34:877–889
- Hamm PC (1974) Discovery, development, and current status of the chloroacetamide herbicides. *Weed Sci* 22:541–545
- Hartley MJ (1994) Dalapon resistant individuals in a susceptible population of Chilean needle grass. Pages 75–79 in Proceedings of the New Zealand Plant Protection Conference, Waitangi, New Zealand, August 9–11, 1994
- Haslam TM, Kunst L (2013) Extending the story of very-long-chain fatty acid elongation. *Plant Sci* 210:93–107
- Haslam TM, Kunst L (2020) *Arabidopsis* ECERIFERUM2-LIKEs are mediators of condensing enzyme function. *Plant Cell Physiol* 61:2126–2138
- Heap I (2023) The International Herbicide-Resistant Weed Database. <http://www.weedscience.org>. Accessed: August 30, 2023
- Heydens WF, Lamb IC, Wilson AG (2010) Chloracetanilides. Pages 1753–1769 in Krieger R, ed. *Hayes' Handbook of Pesticide Toxicology*. London: Academic Press
- Huai D, Xue X, Li Y, Wang P, Li J, Yan L, Chen Y, Wang X, Liu N, Kang Y, Wang Z, Huang Y, Jiang H, Lei Y, Liao B (2020) Genome-wide identification of peanut KCS genes reveals that *AhKCS1* and *AhKCS28* are involved in regulating VLCFA contents in seeds. *Front Plant Sci* 11:406
- Hwang J-I, Norsworthy JK, Carvalho-Moore P, Barber LT, Butts TR, McElroy JS (2023) Exploratory analysis on herbicide metabolism and very-long-chain fatty acid production in metolachlor-resistant Palmer amaranth (*Amaranthus palmeri* S. Wats.). *J Agric Food Chem* 71:6014–6022

- Jhala AJ, Malik MS, Wills JB (2015) Weed control and crop tolerance of micro-encapsulated acetochlor applied sequentially in glyphosate-resistant soybean. *Can J Plant Sci* 95:973–981
- Joubès J, Raffaele S, Bourdenx B, Garcia C, Laroche-Traineau J, Moreau P, Domergue F, Lessire R (2008) The VLCFA elongase gene family in *Arabidopsis thaliana*: phylogenetic analysis, 3D modelling and expression profiling. *Plant Mol Biol* 67:547–566
- Juliano LM, Casimero MC, Llewellyn R (2010) Multiple herbicide resistance in barnyardgrass (*Echinochloa crus-galli*) in direct-seeded rice in the Philippines. *Int J Pest Manage* 56:299–307
- Kasahara T, Matsumoto H, Hasegawa H, Koyama K, Takeuchi T (2019) Characterization of very long chain fatty acid synthesis inhibition by ipfencarbazone. *J Pestic Sci* 44:20–24
- Kaur T, Bhullar MS, Kaur S (2019) Control of herbicide resistant *Phalaris minor* by pyroxasulfone in wheat. *Indian J Weed Sci* 51:123–128
- Kern AJ, Colliver CT, Maxwell BD, Fay PK, Dyer WE (1996) Characterization of wild oat (*Avena fatua* L.) populations and an inbred line with multiple herbicide resistance. *Weed Sci* 44:847–852
- Kern AJ, Jackson LL, Dyer WE (1997) Fatty acid and wax biosynthesis in susceptible and triallate-resistant *Avena fatua* L. *Pestic Sci* 51:21–26
- Kern AJ, Myers TM, Jasieniuk M, Murray BG, Maxwell BD, Dyer WE (2002) Two recessive gene inheritance for triallate resistance in *Avena fatua* L. *J Hered* 93:48–50
- Keshtkar E, Mathiassen SK, Moss SR, Kudsk P (2015) Resistance profile of herbicide-resistant *Alopecurus myosuroides* (black-grass) populations in Denmark. *Crop Prot* 69:83–89
- Kido T, Okita H, Okamura M, Takeuchi T, Morita K (2016) Development of a rice herbicide, ipfencarbazone. *J Pestic Sci* 41:113–119
- Kohler EA, Branham BE (2002) Site of uptake, absorption, translocation, and metabolism of ethofumesate in three turfgrass species. *Weed Sci* 50: 576–580
- Kouame KBJ, Bertucci MB, Savin MC, Bararpour T, Steckel LE, Butts TR, Roma-Burgos N (2022) Resistance of Palmer amaranth (*Amaranthus palmeri*) to S-metolachlor in the midsouthern United States. *Weed Sci* 70:380–389
- Krähmer H, Dücker R, Beffa R, Babczinski P (2019) Herbicides disturbing the synthesis of very-long-chain fatty acids. Pages 351–363 in Jeschke P, Witschel M, Krämer W, Schirmer U, eds. *Modern Crop Protection Compounds*, 3rd ed. Weinheim, Germany: Wiley-VCH Verlag GmbH & Co. KGaA
- Kudou T, Tanima D, Masuzawa Y, Yano T, inventors; Nissan Chemical Ind Ltd, assignee (2010) Ortho-substituted haloalkylsulfonanilide derivative herbicides. World Patent WO2010026989. 2010 Nov 3
- Lamoureux GL, Rusness DG (1986) Tridiphane [2-(3,5-dichlorophenyl)-2-(2,2,2-trichloroethyl)oxirane] an atrazine synergist: enzymatic conversion to a potent glutathione S-transferase inhibitor. *Pestic Biochem Physiol* 26:323–342
- Lin J, Li Y, Hu X, Chi W, Zeng S, Xu J (2021) Discovery of novel 3-[[[(5,5-dimethyl-4,5-dihydroisoxazol-3-yl)sulfonyl]methyl]benzo[d]isoxazole analogs as promising very long chain fatty acids inhibitors. *J Heterocyclic Chem* 58:226–240
- Liu M, Hulting AG, Mallory-Smith C (2016) Characterization of multiple herbicide-resistant Italian ryegrass (*Lolium perenne* ssp. *multiflorum*) populations from winter wheat fields in Oregon. *Weed Sci* 64:331–338
- Ma HJ, Li YH, Zhao QF, Zhang T, Xie RL, Mei XD, Ning J (2010) Synthesis and herbicidal activity of novel N-(2,2,2)-trifluoroethylpyrazole derivatives. *J Agric Food Chem* 58:4356–4360
- Mallory-Smith C, Retzinger EJ (2017) Revised classification of herbicides by site of action for weed resistance management strategies. *Weed Technol* 17:605–619
- Mangin AR, Hall LM, Beckie HJ (2016) Triallate-resistant wild oat (*Avena fatua* L.): unexpected resistance to pyroxasulfone and sulfentrazone. *Can J Plant Sci* 97:20–25
- Matthes B, Schmalfuß J, Böger P (1998) Chloroacetamide mode of action. II: Inhibition of very-long-chain fatty acid synthesis in higher plants. *Z Naturforsch* 53c:1004–1011
- Millar AA, Clemens S, Zachgo S, Giblin EM, Taylor DC, Kunst L (1999) *CUT1*, an *Arabidopsis* gene required for cuticular wax biosynthesis and pollen fertility, encodes a very-long-chain fatty acid condensing enzyme. *Plant Cell* 11:825–838
- Millar AA, Kunst L (1997) Very-long-chain fatty acid biosynthesis is controlled through the expression and specificity of the condensing enzyme. *Plant J* 12:121–131
- Moser H, Rihs G, Sauter HP, Böhner B (1983) Atropisomerism, chiral centre and activity of metolachlor. Pages 315–320 in Doyce P, Fujita T, eds. *Pesticide Chemistry: Human Welfare and Environment*. Oxford: Pergamon Press
- Nakatani M, Yamaji Y, Honda H, Uchida Y (2016) Development of the novel pre-emergence herbicide pyroxasulfone. *J Pest Sci* 41:107–112
- O'Connell PJ, Harms CT, Allen JR (1998) Metolachlor, S-metolachlor and their role within sustainable weed-management. *Crop Prot* 17:207–212
- O'Donovan JT, Sharma MP, Harker KN, Maurice D, Baig MN, Blackshaw RE (1994) Wild oat (*Avena fatua*) populations resistant to triallate are also resistant to difenzoquat. *Weed Sci* 42:195–199
- Parker DC, Simmons FW, Wax LM (2005) Fall and early preplant application timing effects on persistence and efficacy of acetamide herbicides. *Weed Technol* 19:6–13
- Pike DR, McGlamery MD, Knake EL (1991) A case study of herbicide use. *Weed Technol* 3:639–646
- Pillai P, Davis DE, Truelove B (1979) Effects of metolachlor on germination, growth, leucine uptake, and protein synthesis. *Weed Sci* 27: 634–637
- Post-Beittenmiller D (1996) Biochemistry and molecular biology of wax production in plants. *Annu Rev Plant Physiol* 47:405–430
- Ramasamy S, McLaren DA, Pritchard G, Officer D, Bonilla J, Preston C, Lawrie AC (2008a) 2,2 DPA resistance in giant parramatta grass (*Sporobolus fertilis*). Pages 71–73 in *Proceedings of the 16th Australian Weeds Conference*. Cairns, Australia, May 18–22, 2008
- Ramasamy S, Pritchard G, McLaren DA, Bonilla J, Preston C, Lawrie AC (2008b) Field survey of flupropanate-resistant *Nassella trichotoma* in Victoria. Pages 18–22 in *Proceedings of the 16th Australian Weeds Conference*. Cairns, Australia, May 18–22, 2008
- Rangani G, Noguera M, Salas-Perez R, Benedetti L, Roma-Burgos N (2021) Mechanism of resistance to S-metolachlor in Palmer amaranth. *Front Plant Sci* 12:652581
- Rashid A, O'Donovan JT, Khan AA, Blackshaw RE, Harker KN, Pharis RP (1998) A possible involvement of gibberellin in the mechanism of *Avena fatua* resistance to triallate and cross-resistance to difenzoquat. *Weed Res* 38:461–466
- Rauch TA, Thill DC, Gersdorf SA, Price WJ (2010) Widespread occurrence of herbicide-resistant Italian ryegrass (*Lolium multiflorum*) in Northern Idaho and Eastern Washington. *Weed Technol* 24:281–288
- Riar DS, Norsworthy JK, Johnson DB, Starkey CE, Lewis A (2011) Efficacy and cotton tolerance to Warrant herbicide. Fayetteville: Arkansas Agricultural Experiment Station. Research Series 602:30–35
- Schmalfuß J, Matthes B, Mayer P, Böger P (1998) Chloroacetamide mode of action. I: Inhibition of very-long-chain fatty acid synthesis in *Scenedesmus acutus*. *Z Naturforsch* 53c:995–1003
- Selby TP, Smith BT, Stevenson TM, inventors; EIDP Inc, assignee (2008) Substituted pyridine n-oxide herbicides. World Patent WO2008100426. 2008 Feb 8
- Senseman SA (2007) *The Herbicide Handbook 2007*. Westminster, CO: Weed Science Society of America 458 p
- Shaner DL, Brunk G, Belles D, Westra P, Nissen S (2006) Soil dissipation and biological activity of metolachlor and S-metolachlor in five soils. *Pest Manag Sci* 7:617–623
- Shaner DL, Jachetta JJ, Senseman S, Burke I, Hanson B, Jugulam M, Tan S, Reynolds J, Strek H, McAllister R, Green J, Glenn B, Turner P, Pawlak J (2014) *Herbicide Handbook*. 10th ed. Lawrence, KS: Weed Science Society of America. 513 p
- Sherwani SI, Arif IA, Khan HA (2015) Modes of action of different classes of herbicides. Pages 165–186 in *Herbicides, Physiology of Action, and Safety*. doi:10.5772/61779
- Smith BT, Selby TP, Stevenson TM, Clark DA, Taggi AE, inventors; E. I. Du Pont De Nemours and Company, assignee (2009) Herbicidal dihydro oxo six-membered azinyl isoxazolines. World Patent WO2009158258 A1. 2009

- Dec 30. <https://patents.google.com/patent/WO2009158258A1> Accessed: November 21, 2023
- Strom SA, Gonzini LC, Mitsardarfer C, Davis AS, Riechers DE, Hager AG (2019) Characterization of multiple herbicide-resistant waterhemp (*Amaranthus tuberculatus*) populations from Illinois to VLCFA-inhibiting herbicides. *Weed Sci* 67:369–379
- Strom SA, Hager AG, Concepcion JCT, Seiter NJ, Davis AS, Morris JA, Kaundun SS, Riechers DE (2021) Metabolic pathways for S-metolachlor detoxification differ between tolerant corn and multiple-resistant waterhemp. *Plant Cell Physiol* 62:1770–1785
- Strom SA, Hager AG, Seiter NJ, Davis AS, Riechers DE (2020) Metabolic resistance to S-metolachlor in two waterhemp (*Amaranthus tuberculatus*) populations from Illinois, USA. *Pest Manag Sci* 76:3139–3148
- Suzukawa AK, Bobadilla LK, Mallory-Smith C, Brunharo CA (2021) Non-target-site resistance in *Lolium* spp. globally: a review. *Front Plant Sci* 11:609209
- Székács A (2021) Herbicide mode of action. Pages 41–86 in Mesnage R, Zaller JG, eds. *Herbicides*. Amsterdam: Elsevier
- Takahashi H, Schmalfuß J, Ohki A, Hosokawa A, Tanaka A, Sato Y, Matthes B, Böger P, Wakabayashi K (2002) Inhibition of very long chain fatty acid formation by indanofan, 2-[2-(3-Chlorophenyl)oxiran-2-ylmethyl]-2-ethylindan-1,3-dione, and its relatives. *Z Naturforsch C* 57:72–74
- Takamasa F, Mama I, Masato O, Daisuke T, Yoshihiko N (2023) Dimesulfazet – discovery and mode of action of a novel rice paddy herbicide. Page 6 in Proceedings of the 15th IUPAC International Congress of Crop Protection Chemistry. New Delhi, India, March 14–17, 2023
- Tanetani Y, Fujioka T, Horita J, Kaku K, Shimizu T (2011a) Action mechanism of a novel herbicide, fenoxasulfone. *J Pestic Sci* 36:357–362
- Tanetani Y, Fujioka T, Kaku K, Shimizu T (2011b) Studies on the inhibition of plant very-long-chain fatty acid elongase by a novel herbicide, pyroxasulfone. *J Pestic Sci* 36:221–228
- Tanetani Y, Ikeda M, Kaku K, Shimizu T, Matsumoto H (2013) Role of metabolism in the selectivity of a herbicide, pyroxasulfone, between wheat and rigid ryegrass seedlings. *J Pestic Sci* 38:152–156
- Tanetani Y, Kaku K, Kawai K, Fujioka T, Shimizu T (2009) Action mechanism of a novel herbicide, pyroxasulfone. *Pestic Biochem Physiol* 95:47–55
- Trenkamp S, Martin W, Tietjen K (2004) Specific and differential inhibition of very-long-chain fatty acid elongases from *Arabidopsis thaliana* by different herbicides. *Proc Natl Acad Sci USA* 101:11903–11908
- Tresch S, Heilmann M, Christiansen N, Looser R, Grossmann K (2012) Inhibition of saturated very-long-chain fatty acid biosynthesis by mefluidide and perfluidone, selective inhibitors of 3-ketoacyl-CoA synthases. *Phytochemistry* 76:162–171
- Umetsu N, Shirai Y (2020) Development of novel pesticides in the 21st century. *J Pest Sci* 45:54–74
- [US-EPA] U.S. Environmental Protection Agency (1994) Pesticide Product Label, Acetochlor EC Herbicide https://www3.epa.gov/pesticides/chem_sea_rch/ppls/066478-00002-19940311.pdf. Accessed: February 23, 2023
- [US-EPA] U.S. Environmental Protection Agency (1998) Propachlor: Reregistration Eligibility Decision (RED) <https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=1337bff37f41f1428260154a5907303b7d9514cb>. Accessed: March 1, 2023
- [USDA-NASS] U.S. Department of Agriculture–National Agricultural Statistics Service (2019) 2018 Agricultural Chemical Use Survey for Peanuts. NASS Highlights. https://www.nass.usda.gov/Surveys/Guide_to_NASS_Surveys/Chemical_Use/2018_Peanuts_Soybeans_Corn/ChemUseHighlights_Peanuts_2018.pdf. Accessed: February 21, 2023
- [USDA-NASS] U.S. Department of Agriculture–National Agricultural Statistics Service (2020a) 2019 Agricultural Chemical Use Survey for Sorghum. NASS Highlights. https://www.nass.usda.gov/Surveys/Guide_to_NASS_Surveys/Chemical_Use/2019_Field_Crops/chem-highlights-sorghum-2019.pdf. Accessed: February 21, 2023
- [USDA-NASS] U.S. Department of Agriculture–National Agricultural Statistics Service (2020b) 2020 Agricultural Chemical Use Survey for Soybeans. <https://quickstats.nass.usda.gov/results/01439D0D-C74B-37E6-95CA-2F65EF91B816#02F3F29F-5252-34AF-BAA1-9CC4B35F5711>. Accessed: February 22, 2023
- [USDA-NASS] U.S. Department of Agriculture–National Agricultural Statistics Service (2021a) 2021 Agricultural Chemical Use Survey for Corn. <https://quickstats.nass.usda.gov/results/600680DE-CCF5-3467-8459-77A2E59C3141#AFE78139-6D34-3AD2-9CF0-F9192DB02AA6>. Accessed: February 22, 2023
- [USDA-NASS] U.S. Department of Agriculture–National Agricultural Statistics Service (2021b) 2020 Agricultural Chemical Use Survey for Soybeans. NASS Highlights. https://www.nass.usda.gov/Surveys/Guide_to_NASS_Surveys/Chemical_Use/2020_Soybeans/soybean-chem-highlights.pdf. Accessed: February 21, 2023
- [USDA-NASS] U.S. Department of Agriculture–National Agricultural Statistics Service (2021c) 2020 Agricultural Chemical Use Survey for Vegetable Crops. NASS Highlights. https://www.nass.usda.gov/Surveys/Guide_to_NASS_Surveys/Chemical_Use/2020_Vegetables/ChemHighlights-Veg.pdf. Accessed: February 21, 2023
- [USDA-NASS] U.S. Department of Agriculture–National Agricultural Statistics Service (2022) 2021 Agricultural Chemical Use Survey for Corn. NASS Highlights. https://www.nass.usda.gov/Surveys/Guide_to_NASS_Surveys/Chemical_Use/2021_Field_Crops/chemhighlights-corn.pdf. Accessed: February 21, 2023
- [USGS] U.S. Geological Survey (2022) Estimated Annual Agricultural Pesticide Use for Acetochlor. https://water.usgs.gov/nawqa/pnsp/usage/maps/show_map.php?year=2019&map=ACETOCHLOR&hilo=L&disp=Acetochlor. Accessed: February 22, 2023
- [USGS-NAWQA] U.S. Geological Survey National Water-Quality Assessment Project (2022) Estimated Annual Agricultural Pesticide Use. https://water.usgs.gov/nawqa/pnsp/usage/maps/compound_listing.php?year=2019&hilo=L. Accessed: February 22, 2023
- [WHO] World Health Organization (1988) Thiocarbamate pesticides: a general introduction. Geneva: World Health Organization
- Wieben CM (2019) Estimated Annual Agricultural Pesticide Use by Major Crop or Crop Group for States of the Conterminous United States, 1992–2017 (ver. 2.0, May 2020): U.S. Geological Survey data release. <https://doi.org/10.5066/P9HHG3CT>. Accessed: February 22, 2023
- [WSSA] Weed Science Society of America (2021) WSSA-Herbicide Site of Action (SOA) Classification List. https://wssa.net/wp-content/uploads/WSSA-Herbicide-SO_WSSA_20210505.xlsx. Accessed: March 2, 2023
- [WSSA] Weed Science Society of America (2017) WSSA survey ranks most common and most troublesome weeds in broadleaf crops, fruits, and vegetables. <https://wssa.net/2017/05/wssa-survey-ranks-most-common-and-most-troublesome-weeds-in-broadleaf-crops-fruits-and-vegetables/>. Accessed: August 30, 2023
- Weisshaar H, Böger P (1987) Primary effects of chloroacetamides. *Pestic Biochem Physiol* 28:286–293
- Wilson RG (1984) Accelerated degradation of thiocarbamate herbicides in soil with prior thiocarbamate herbicide exposure. *Weed Sci* 32: 264–268
- Yun MS, Yogo Y, Miura R, Yamasue Y, Fischer AJ (2005) Cytochrome P-450 monooxygenase activity in herbicide-resistant and-susceptible late watergrass (*Echinochloa phyllopogon*). *Pestic Biochem Phys* 83:107–114
- Zollinger RK (2011) Summary of new information in the 2011 Weed Control Guide. Fargo: North Dakota State University Extension Service. https://library.ndsu.edu/ir/bitstream/handle/10365/14266/text_summary.pdf?sequence=23&isAllowed=y. Accessed: March 2, 2023