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Environmental Fate and Effects of Dichloroacetamide Herbicide Safeners: "Inert" yet Biologically Active Agrochemical Ingredients

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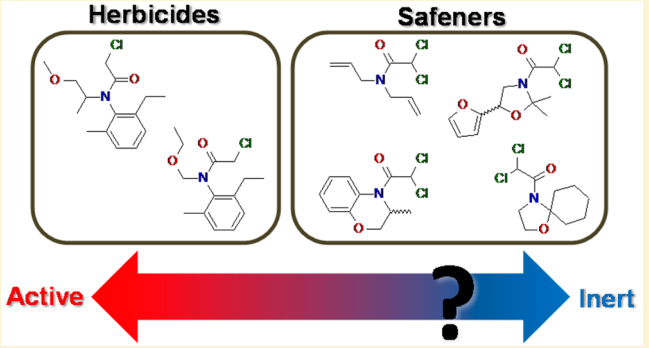
Supporting Information

ABSTRACT: Safeners are included in many commercial herbicide formulations to selectively protect crops from injury induced by active ingredients. Despite their bioactivity, safeners are classified as inert from a regulatory perspective, and as such, safeners have received minimal attention in the peer-reviewed literature regarding their environmental fate and effects. Herein, we review what is known about the uses, physicochemical properties, environmental transformations, and (eco)toxicological effects of dichloroacetamide safeners, which represent one of the most commonly used safener classes (estimated use of >2 × 10^6 kg/year in the United States). We particularly highlight transformation pathways that may enhance biological activity and/or persistence; for example, limited studies suggest dichloroacetamides can transform via dechlorination into products with increased bioactivity. We also identify several research needs to improve our understanding of the environmental fate and potential risks of this overlooked agrochemical class, which in turn will enhance the efficacy and safety of future herbicide safener formulations.



INTRODUCTION

Since 2001, global herbicide use has approached 10^9 kg of active ingredient per year.[1] A key challenge associated with herbicide use is selectivity.[2,3] In addition to killing the targeted weed species, most herbicides can also exert toxicity on crops.[4−6] This collateral damage can be mitigated, however, by adding a safener to herbicide formulations.[5,6] Safeners (formerly called antidotes, antagonists, or protectants) are chemicals that enhance the ability of crops (but not weeds) to detoxify herbicides.[4,7] Safeners can, for example, promote *in vivo* transformations of herbicides into products with less or no appreciable herbicidal activity (for reviews on modes of action, see refs 3, 5, and 8−12). Safeners are typically applied as either seed treatments (e.g., with sorghum), water applications (rice), or spray formulations (corn and other cereals) containing one or more herbicides and adjuvants.[3,4,6]

Since their serendipitous discovery in the 1940s, safeners have emerged as important crop protection products.[13] Commercial use of safeners began in 1971 with the development of 1,8-naphthalic anhydride,[3,4,8,13] and to date, approximately 20 safeners have been used in commercial herbicide formulations.[6,14] These safeners represent a variety of compound classes (Table 1) and include structural motifs (e.g., chloroacetamides, oximes, and thiazoles) also present in some herbicide active ingredients.[15]

Despite recent advances in herbicide-tolerant crops as a potential alternative, current evidence suggests that safeners will continue to play an important role in herbicide products for the foreseeable future. First, including an established safener in a new herbicide formulation can be more cost-effective than imparting herbicide tolerance to crops via genetic modifications.[6] In addition to cost, consumer reservations toward genetically modified crops may also favor continued use of safeners. To wit, in 2011, 30% of all herbicide sales were associated with products containing a safener.[6]

Paradoxically, safeners are both "inert" (from a regulatory perspective[16]) and "active" (from a biological perspective[6,17]). The U.S. Federal Insecticide, Fungicide, and Rodenticide Act requires extensive scrutiny of active ingredients, defined as

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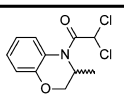
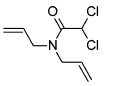
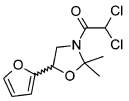
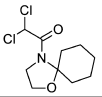
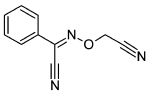
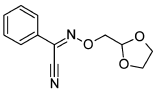
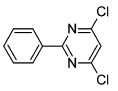
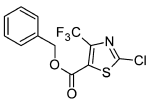
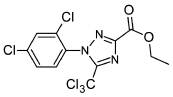
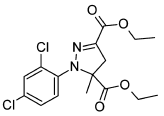
Table 1. Examples of Commercial Herbicide Safeners and Associated Crops[a]

Class	Safener	Crop
Dichloroacetamides	 benoxacor	corn
	 dichlormid	corn
	 furilazole	cereal crops
	 AD-67	corn
Oxime ethers	 cyometrinil	sorghum
	 oxabetrinil	sorghum
Other classes	 1,8-naphthalic anhydride	corn
	 fenclorim	rice
	 flurazole	sorghum
	 fenchlorazole-ethyl	cereal crops
	 mefenpyr-diethyl	cereal crops

[a]For a more comprehensive list of commercial safeners, see refs 6 and 14.

constituents that "prevent, destroy, repel, or mitigate any pest".[16,18] Because the activity of safeners is not directed toward pests, they are classified as "inert" (or "other") ingredients in the United States and in several other countries.[3,16,19,20] As the U.S. Environmental Protection Agency notes, "The term "inert" is not intended to imply nontoxicity; the ingredient may or may not be chemically active."[21] The fact that safeners can, by design, alter the biochemistry of crops and impart protection from herbicide-induced injury supports the biologically active character of these agrochemicals. Although registration requirements of safeners in the United States are somewhat less stringent than those of herbicides,[19] manufacturers are still required to submit environmental fate and effects data, typically including measurements of hydrolysis, soil adsorption, photolysis, stability in soils, and toxicity (acute and chronic).[22−25] In the European Union, courts have considered whether safeners should retain their "inert" classification.[26] Recent European Union policy changes have, however, largely harmonized the conditions required for approval of safeners and active substances.[27]

Although safeners possess structures similar to those of some herbicides, their environmental fate[28−33] and effects[34−37] have received minimal attention in the peer-reviewed literature relative to the extensive literature covering active ingredients. We posit that the classification of safeners as "inert" has contributed to the paucity of environmental research on this largely overlooked class of agrochemicals. A more comprehensive understanding of the environmental fate and effects of safeners is important because these compounds are extensively used, are biologically active, and are introduced into the environment in formulations containing additional bioactive agents, leaving open the possibility of synergistic and/or antagonistic effects. In addition, previous (albeit limited) studies[29,30] suggest some safeners can transform in the environment to yield products with increased biological activity relative to that of the parental safeners. Herein, we review the peer-reviewed and gray literature (e.g., reports released from chemical manufacturers and governmental agencies) for what is known about the physicochemical properties, environmental transformations, and (eco)toxicological effects of safeners. As several of the most commonly used safeners are dichloroace-

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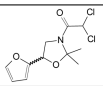
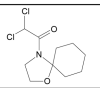
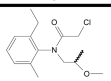
Table 2. Physical and Chemical Properties of Dichloroacetamide Safeners and Herbicidal Co-Formulants

	Common Name	Additional Identifier	Structure	Log K_{ow}[a]	C_w^{sat} (mg/L)[a]	Log K_{aw}[a]	Aerobic DT_{50}[b]	Anaerobic DT_{50}[b]	Typical Herbicidal Co-Formulant
Safeners	dichlormid	R-25788		1.84	1070	-4.87	8 days[25]	not available	acetochlor
	furilazole	MON 13900		2.12	254	-8.42	13 days[24]	not available	acetochlor
	benoxacor	CGA-154281		2.70	103	-5.51	49 days[22]	70 days[22]	metolachlor
	AD-67	MON 4660		3.19	43	-7.29	18 days[23]	not available	acetochlor
Herbicides	metolachlor	--		2.90	51	-6.43	26 days[42]	37 days[42]	--
	acetochlor	--		3.03	47	-6.04	11 days[43]	19 days[43]	--

[a]Octanol–water (K_{ow}) and air–water (K_{aw}) partition coefficients and water solubility (C_w^{sat}, 25 °C) data from ref 44. [b]DT_{50} denotes the median dissipation half-life of the parent compound in soil.

tamides,[3,6,8] this review will focus on this class of safener, although many of the research priorities we identify for dichloroacetamides can be extended more generally to other safener classes (Table 1).

ESTIMATES OF ANNUAL USE

Dichloroacetamide safeners have been in commercial use for more than four decades[38] and are currently used on several continents, including North America, Europe, Africa, and Asia.[6,39] Dichloroacetamide safeners are typically applied as pre-emergence spray formulations that also contain a chloroacetamide herbicide (Table 2).[20,30] Benoxacor, for example, is commonly paired with *S*-metolachlor in commercial formulations such as Dual II Magnum (Syngenta).[5,7] Dichlormid is often paired with acetochlor (e.g., Surpass EC, Dow AgroSciences) and can also be combined with thiocarbamate herbicides.[2,5,7] Furilazole is commonly paired with acetochlor (e.g., Degree, Monsanto).[2−5,7] AD-67 is frequently sold separately from its common herbicidal partner (acetochlor); formulations containing AD-67 are typically prepared by users.

Unlike herbicide active ingredients, usage data for safeners (and other inert constituents) are generally not compiled. Estimates can, however, be made on the basis of the available data for herbicide active ingredients.[40] In 2012 (the most recent year for which data are available), at least 16 million kg of *S*-metolachlor was applied in the United States.[40] Approximately 70% of *S*-metolachlor-containing formulations include benoxacor or dichlormid as a safener,[41] and as a first approximation, we assume these formulations have equal usage (mass per year). On average, benoxacor or dichlormid is present in these formulations at 14% (by mass) relative to *S*-metolachlor.[45,46] These data suggest a total usage of 1.6×10^6 kg/year (benoxacor + dichlormid) for formulations containing *S*-metolachlor. This estimate is likely to be conservative because in addition to *S*-metolachlor, benoxacor and dichlormid are coformulated with several additional active ingredients (e.g., racemic metolachlor, halosulfuron-methyl, and acetochlor). Moreover, benoxacor and dichlormid are not the only dichloroacetamide safeners currently in use (Table 2). Accordingly, the total annual use for all dichloroacetamide safeners is estimated to be $>2 \times 10^6$ kg in the United States, surpassing that of many herbicide active ingredients.[40] As the United States accounts for 25% of herbicide use worldwide,[1] global use of dichloroacetamide safeners is predicted to exceed 8×10^6 kg/year. Between 2008 and 2012 (the most recent year for which data are available), U.S. applications of *S*-metolachlor increased by 40%, with annual use increasing each year in this five-year period.[40] Over the same period, annual use of other herbicides commonly paired with dichloroacetamide safeners (e.g., racemic metolachlor and acetochlor) also increased.[40] Accordingly, to the extent that safener use tracks with use of herbicidal coformulants, applications of dichloroacetamide safeners are also likely to have trended upward in recent years.

PHYSICOCHEMICAL PROPERTIES AND ENVIRONMENTAL MOBILITY

Selected physicochemical properties of the four commercial dichloroacetamide safeners are compiled in Table 2. For comparison, properties of two chloroacetamide herbicides (acetochlor and metolachlor) are also included. Air–water partition coefficients for dichloroacetamides are small (log K_{aw} = −8.4 to −4.9),[44] suggesting volatilization is negligible. Given their structural similarity to chloroacetamide herbicides (which have been detected in surface water,[47−49] groundwater,[49−53] and finished drinking water[54,55]), dichloroacetamides are anticipated to be mobile in soils and readily transported into aqueous systems.

Furilazole, for example, was shown to adsorb only modestly to a variety of soils (K_d = 0.79−3.5 mL/g), with the extent of adsorption increasing with clay content and cation exchange capacity.[56] Dichlormid demonstrated an even lower extent of adsorption across several soil types (K_d = 0.25−0.65 mL/g).[57] Soil sorption data for benoxacor and AD-67 do not appear to be available. The larger K_{ow} values of benoxacor and AD-67 suggest these safeners may adsorb to a greater extent onto soils compared to furilazole and dichlormid; however, prior

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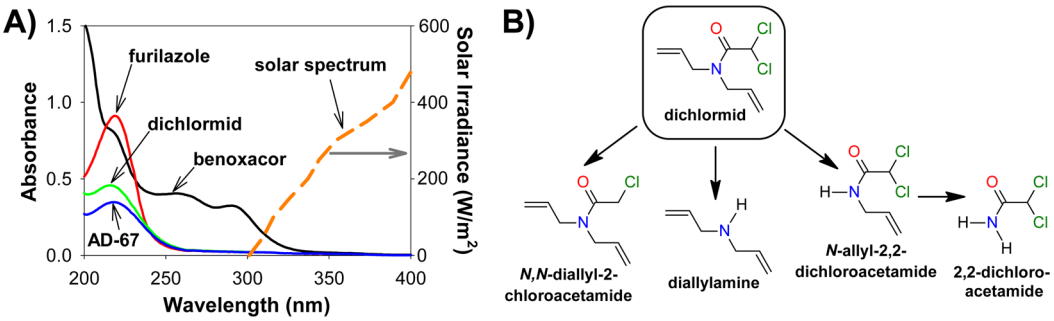

Figure 1. (A) Spectra for the absorbance of dichloroacetamide safeners (50 μM) and solar irradiance at Earth's surface. Wavelengths of maximal absorbance: benoxacor, 257 nm; dichlormid, 214 nm; furilazole, 218 nm; AD-67, 217 nm. (B) Potential photochemical transformation pathways for dichlormid in water irradiated with light at 254 nm (adapted from ref 29).

correlations between K_{ow} and soil–water partitioning coefficients for chloroacetamides have proven to be spurious, likely because of contributions from specific adsorption interactions (e.g., formation of coordination bonds, $\pi-\pi$ charge transfer bonds, and hydrogen bonds between chloroacetamides and environmental sorbents).[58] For comparison, experimental soil K_d values (assuming equilibrium concentrations in solution of 1 μM) for the herbicides metolachlor (2.5–11.77 mL/g) and acetochlor (1.82–10.83 mL/g)[58] are generally greater than those of dichlormid and furilazole, suggesting these safeners will be at least as mobile in soils as chloroacetamide active ingredients. Nevertheless, environmental occurrence data for dichloroacetamide safeners have, to the best of our knowledge, not been reported.

■ ABIOTIC TRANSFORMATION PATHWAYS

Dichloroacetamide safeners contain electron-deficient (e.g., dichloroacetyl) and electron-rich (e.g., furanyl, benzoxazinyl, and allyl) moieties that are possible targets for reactions with nucleophiles and electrophiles, respectively. As dichloroacetamides can react with both electron-rich (e.g., complexed ferrous iron) and electron-deficient species (e.g., photogenerated oxidants), these safeners are anticipated to transform in the environment under both oxidizing and reducing conditions.

Hydrolysis. Hydrolysis of dichloroacetamides is very slow. For example, no appreciable loss of dichlormid and furilazole was observed after 4 weeks in sterile buffered solutions at pH 5, 7, and 9 incubated at 25 °C.[59,60] In solutions maintained at pH 9 and 40 °C, slow hydrolysis of dichlormid was observed (~10% loss of parent after 29 days).[59] Metabolites of dichloroacetamides in which the dichloroacetyl group is altered may, however, be more susceptible to hydrolysis than the parent compounds are.[24]

Photochemical Transformations. From absorption spectra in water (Figure 1A), benoxacor exhibits a broad UV absorbance band that extends beyond 300 nm, generating sufficient overlap with the solar spectrum to permit direct photolysis (assuming sufficient photoefficiencies). Other dichloroacetamides exhibit maximal absorbance wavelengths (λ_{max} ~ 215 nm) outside of those available in sunlight, thus suggesting little to no direct photolysis in sunlit waters, although it may be relevant in engineered systems using UV (254 nm) light for disinfection. All dichloroacetamide safeners, including benoxacor, contain functionalities likely susceptible to reaction with photogenerated reactive oxygen species [ROS, including hydroxyl radical ($OH^{\bullet}$) from nitrate photolysis] or triplet state dissolved organic matter (DOM*); therefore, some reaction via indirect photolysis is anticipated.

To date, the most detailed photolysis study examined dichlormid transformation during irradiation with 254 nm light,[29] reporting direct photolysis half-lives and identifying major transformation products. The half-life for dichlormid was ~10 min (under a 125 W high-pressure mercury lamp), with transformation proceeding via dealkylation, photoassisted hydrolysis, and dechlorination (Figure 1B). Notably, dechlorination generates a previously used herbicide active ingredient, *N*,*N*-diallyl-2-chloroacetamide (CDAA, also termed allidochlor).[29] The authors also reported limited to no transformation of dichlormid upon exposure to light above 290 nm,[29] consistent with dichlormid's absorbance spectrum and suggesting negligible direct photolysis in sunlit surface waters.

Additional insights into the photochemical reactivity of dichloroacetamide safeners can be derived from (non-peer-reviewed) manufacturer reports, although such information is often incomplete and/or nonquantitative. For example, despite the likelihood of benoxacor direct photolysis, there is no mention of photodegradation in its pesticide tolerance report.[22] Similarly, there is no mention of dichlormid photodegradation in its manufacturer's report,[25] while "only a small amount" of photodegradation of AD-67 is reported.[23]

Manufacturer's reports for furilazole provide a more detailed treatment, considering phototransformation not only in homogeneous aqueous solutions but also in heterogeneous soil systems.[61,62] At pH 7, irradiation with simulated sunlight (λ > 290 nm) produced an experimental half-life equivalent to 29.9 days under natural sunlight.[61] Further, a complex mixture of reaction products, including major product *N*-(dichloroacetyl)glycine, was reported for the system nearly 6 days after irradiation, and corresponding formation mechanisms were proposed.[61] The half-life of furilazole decreased considerably to 7.85 h (sunlight equivalent) in the presence of 25 mM humic acid, consistent with indirect photolysis pathways, and *N*-(dichloroacetyl)glycine remained the major (23.5%) identifiable product ~3 days after irradiation.[61] In complementary work, furilazole was also applied to a silty clay loam (3.5% organic matter content) and subsequently irradiated, with extracted soil samples suggesting a half-life of 8 days, presumably because of a combination of direct and indirect photolysis.[62] To the best of our knowledge, this work with furilazole represents the only investigation of indirect photolysis for any of the dichloroacetamide safeners.

Dark, Abiotic Redox Reactions. Dichloroacetamide safeners can undergo abiotic hydrogenolysis (reductive dechlorination) in slurries containing Fe^{II}-amended iron

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(hydr)oxides.[30] In these systems, benoxacor, dichlormid, and AD-67 were reduced to their monochloro analogues. For reactions of dichlormid, two additional monochloro products [**I** and **II** (Figure 2)] were generated in parallel to the anticipated

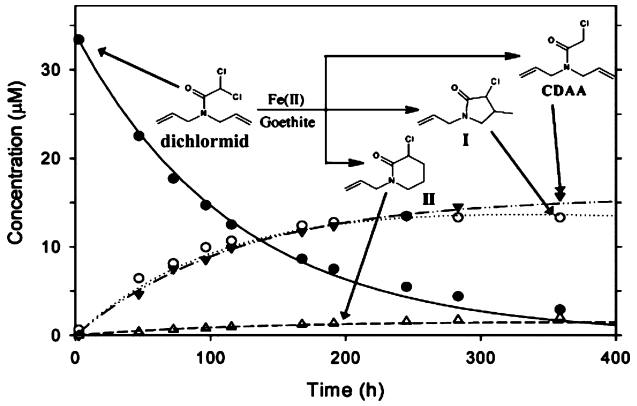

Figure 2. Conversion of dichlormid (a safener) into CDAA (a herbicide) and additional products (**I** and **II**) in Fe^{II}-amended goethite slurries at pH 6.6 and 21 ± 1 °C (adapted from ref 30).

monochloro product (CDAA), presumably via intramolecular cyclization reactions.[30] Reactions of the *N*,*N*-di-*n*-propyl analogue of dichlormid yielded only the anticipated (acyclic) hydrogenolysis product. Accordingly, the allyl groups of dichlormid appear likely to influence product distributions (relative to other dichloroacetamides) under iron reducing conditions (e.g., in soils[63] and aquifers[64]).

In addition to reactions with reducing agents, reactions with oxidizing agents may also influence the fate of dichloroacetamide safeners. Consistent with experimental observations of chloroacetamide herbicides,[65−67] electron-rich moieties of dichloroacetamide safeners (e.g., aromatic and allyl groups) are likely susceptible to reactions with strong oxidants associated with water disinfection, including free chlorine, free bromine, ozone, and $OH^{\bullet}$ generated during advanced oxidation processes.

BIOTRANSFORMATION

In general, dichloroacetamide safeners are extensively metabolized in the environment, and there appears to be significant overlap in the metabolites formed in nontarget plants[28,68−70] and animals[28,70] as well as degradation products observed in soil[28] and natural waters;[70] however, significant knowledge gaps remain. Figure 3 summarizes known biotransformation pathways using dichlormid as an example. The dichloroacetamide moiety of other safeners is expected to be metabolized similarly, although significant differences are anticipated for biotransformations associated with the various N-functionalities.

Biotransformation in Soil. Studies comparing sterile versus nonsterile soil samples demonstrate that microbial action is responsible for the formation of several dichlormid metabolites in soil, including *N*-allyl-2,2-dichloroacetamide, *N*,*N*-diallyl-2-chloroacetamide, and *N*,*N*-diallylacetamide (Figure 3).[28] Reductive dechlorination pathways generating *N*,*N*-diallyl-2-chloroacetamide may be unique to soil metabolism.[28] Aerobic and anaerobic soil biotransformation studies of furilazole demonstrate similarities to the biotransformation of other dichloroacetamide safeners, with 2-hydroxyacetamide and oxamic acid metabolites identified as major products.[71,72] Moreover, methyl sulfide and methyl sulfoxide metabolites of furilazole were also detected in soil.[71] Notably, the methyl sulfoxide moiety of this furilazole metabolite is chiral and thus may be formed stereoselectively via biotic processes.

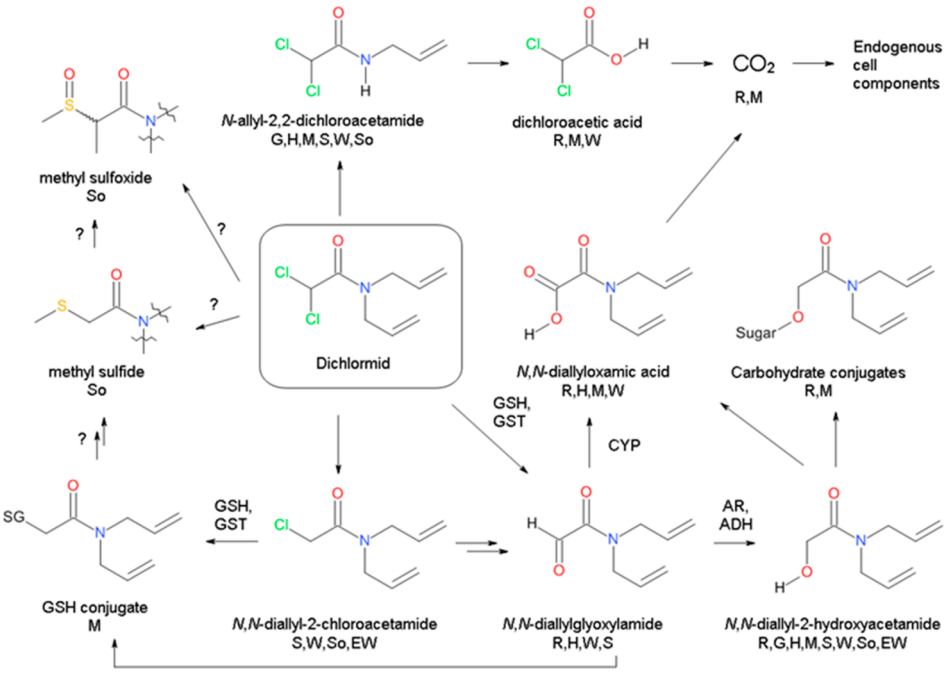

Figure 3. Representative biotransformation pathways of the dichloroacetamide safener dichlormid proceeding via dechlorination or N-dealkylation. Biotransformation products observed in animals (G, goat; H, hen; R, rat), plants (C, carrot; M, maize/corn; S, soy; W, wheat), soil (So), and environmental water (EW, environmental water) are indicated. Abbreviations: ADH, alcohol dehydrogenase; AR, aldehyde reductase; GSH, glutathione; GST, glutathione transferase; CYP, cytochrome P450 enzyme. Question marks denote proposed pathways based on structurally analogous biotransformation products observed for other safeners (see the text for additional discussion).

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Additional Biotransformation Pathways and Products. A disposition study of [^{14}C]dichlormid in male and female rats demonstrates that urine is the major route of excretion, followed by feces and exhalation of $^{14}CO_2$ within 4 days of acute administration of [^{14}C]dichlormid (Figure 3).[28] A small percentage of dichlormid was excreted unchanged in 24 h urine, with slightly higher levels observed in male than in female rats. *N*,*N*-Diallyl-2-hydroxyacetamide and its glucuronide conjugate were major urinary metabolites. Levels of *N*,*N*-diallyl-2-hydroxyacetamide were also sex-dependent, with 4-fold higher levels observed in the urine of female than in male rats. These sex differences in the disposition of dichlormid are not surprising because drug-metabolizing enzymes are expressed in a sex-dependent manner in laboratory animals and humans.[73,74] In addition, *N*,*N*-diallyloxamic acid and dichloroacetic acid were minor metabolites detected in rat urine.

Experiments with rat liver microsomes demonstrate that dichlormid is not appreciably metabolized by cytochrome P450 enzymes.[28] Instead, dichlormid was rapidly metabolized by cytosolic enzymes. *In vitro* studies with rat liver cytosol demonstrated that dichlormid was initially conjugated to glutathione, either by direct reaction with glutathione or via reactions catalyzed by glutathione transferases (GSTs). The resulting glutathione conjugates were rapidly transformed via unknown intermediates to *N*,*N*-diallylglyoxylamide, which subsequently was converted to *N*,*N*-diallyl-2-hydroxyacetamide, *N*,*N*-diallyloxamic acid, and *N*,*N*-diallyl-2-hydroxyacetamide (Figure 3). In addition, the presence of dichloroacetic acid in rat urine demonstrates that dichlormid underwent an N-dealkylation reaction in rats; however, the *N*-allyl-2,2-dichloroacetamide precursor was not detected in urine. CO_2 was the final product of both metabolic pathways and was likely incorporated into endogenous cell components.[28]

Dichloroacetamide safeners also undergo rapid biotransformation in hens and lactating goats by N-dealkylation and dechlorination reactions similar to those described above for rats.[70] While the biotransformation pathways of dichloroacetamide safeners in both species have not been fully characterized, some preliminary observations with dichlormid are noteworthy. Total residue levels of [^{14}C]dichlormid were low in goat milk, suggesting only minor lactational transfer of dichlormid and its metabolites in mammals;[70] however, dichlormid residues accumulated somewhat in eggs.[70] The latter observation raises concerns regarding the potential for developmental exposure to dichlormid and its biotransformation products in other species, including mammals.

TOXICOLOGICAL EFFECTS

Ecotoxicology. Benoxacor appears to be the most studied dichloroacetamide safener within both the gray and peer-reviewed literature followed by furilazole (Supporting Information, Table S1). The available data suggest that, among tested taxa, aquatic species are most sensitive to benoxacor and furilazole. Benoxacor can be classified as highly toxic to aquatic autotrophs[75] based on a lowest acute LC_{50} of 0.63 mg/L for freshwater algae. Surprisingly, benoxacor is also moderately toxic to aquatic animal species with a lowest reported LC_{50} of 1.4 mg/L for the freshwater fish, *Ictalurus punctatus*.[76] Longer duration exposures of benoxacor to aquatic organisms cause significant effects at much lower concentrations. In rainbow trout, for example, benoxacor caused significant decreases in condition index (ratio of actual mass to expected mass for a given length) at 0.016 mg/L with a corresponding no observed effect concentration (NOEC) of 0.004 mg/L.[76] In aquatic invertebrates, a 21 day life cycle study in *Daphnia magna* yielded a NOEC of 0.354 mg/L for effects on adult carapace length.[76] Although fewer data are available for furilazole, available information suggests that it is marginally less toxic than benoxacor with a lowest LC_{50} in freshwater fish of 4.6 mg/L.[76] Similar ecotoxicity data are not available for dichlormid and AD-67.

Benoxacor is the only safener to appear in the peer-reviewed ecotoxicology literature, and its toxicity toward terrestrial invertebrates (*Folsomia candida* and *Poecilus cupreus*), algae (*Selenstrum capricornutum*), cyanophytes (*Anabaena cylindrica*), and vascular plants (*Lemna gibba*) appears to be modest.[34,35] Unfortunately, the literature does not provide insight into the toxicity of benoxacor under more realistic exposure or ecological conditions to what appear to be sensitive aquatic animal species. For example, despite the increasing level of recognition of the effects of chemical mixtures,[77] only one study[78] has explored the cotoxicity of safeners and active ingredients and/or transformation products to aquatic systems. In this study, benoxacor alone was more toxic to *Vibrio fischeri* than when combined with the active ingredient, *S*-metolachlor; however, the complete formulation (Dual Gold Safener) was more toxic than benoxacor with or without *S*-metolachlor.[78] This study, however, provides little insight into the potential effects of active/safener exposures in ecological systems. In the case of a benoxacor/*S*-metolachlor mixture, for example, this combination could conceivably exert strong effects on ecological systems as both animals (benoxacor) and autotrophs (benoxacor and *S*-metolachlor) may be directly and adversely affected.

Mammalian Toxicity. Dichloroacetamide safeners display moderate to low toxicity in rats (Supporting Information, Table S2). *In vitro* studies and quantitative structure−activity relationships (QSAR) suggest safeners, such as benoxacor, are unlikely to cause significant endocrine disruption.[79,80] Moreover, safeners did not cause hemolysis, influence markers of lipid peroxidation, or alter catalase activity in human erythrocytes.[37] Co-exposure to herbicide/safener mixtures (alachlor with dichlormid or acetochlor with dichlormid) did not weaken hemolysis relative to herbicide-only exposures.[37] Further mechanistic studies are needed to determine if, like target species,[7,17] dichloroacetamide safeners also upregulate the expression and activity of enzymes involved in the detoxication of safeners (e.g., GSTs) in mammals and other, nontarget species.

Some evidence suggests that certain safeners may be carcinogenic and/or mutagenic. Several structurally related chloroacetamide herbicides are carcinogens in rats, most likely because of their biotransformation to aromatic anilines and, ultimately, formation of DNA-reactive benzoquinone imines.[81,82] Several dichloroacetamide safeners, such as dichlormid, cannot form analogous carcinogenic intermediates because relevant structural elements are missing. The 3,4-dihydro-3-methyl-2*H*-1,4-benzoxazine moiety of benoxacor is an exception and could potentially be metabolized to a reactive quinone imine metabolite. Indeed, an *in silico* screening study using a QSAR system identified benoxacor as a potential mutagen in the Ames test.[83] Because of the significant data gaps regarding their mammalian toxicity, computational toxicology tools represent one approach to identify safeners and their biotransformation products for further toxicity screening, and

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several safeners have indeed been included in larger QSAR toxicity studies.[79,83−88]

FUTURE RESEARCH NEEDS

Because of their widespread use, biological activity, and ability to transform into products of increased (eco)toxicological concern,[6,29,30] dichloroacetamide safeners can be viewed as contaminants of emerging concern[89] whose properties appear to defy their regulatory classification as "inert".[19] Several research gaps exist regarding the environmental fate and effects of dichloroacetamide (and other classes of) safeners, including the following.

(1) Environmental occurrence in various media, particularly soils, surface waters, and groundwaters. A first priority to improve risk assessment is a reliable estimate of probable environmental concentrations, which will help establish exposure risks. Given the lack of reliable usage data for safeners and the limited usage statistics provided by manufacturers, the best path forward likely involves analytical approaches for quantifying safener concentrations in relevant environmental media, as well as any major known metabolites and transformation products. Current hurdles to such occurrence studies relate to analytical method development, particularly for more complex matrices, as well as the availability of commercial standards for metabolites and transformation products to help assess the degree of safener transformation in different environmental media.

(2) Locations and quantities at which safeners are applied. While usage data are available for some agrochemical classes (e.g., pesticides[40] and antibiotics[90,91]), this information is essentially nonexistent for more emerging or underappreciated agrochemicals (e.g., synthetic growth promoters and safeners). An increased level of cooperation with chemical manufacturers regarding sales or usage data would help facilitate estimates of probable environmental concentrations. Such estimates are valuable in concert with results from occurrence studies, where large differences between estimated and measured environmental concentration would imply a more prominent role for metabolites and transformation products in safener fate and risk assessment.

(3) Variables and mechanisms accounting for "dissipation" times in environmental systems. Although most manufacturer reports provide estimates of safener persistence, the processes responsible for "dissipation" are largely unknown. Quantitative fate and transformation studies are thus merited. Further, dissipation does not necessarily correspond to attenuation of hazard, which hinges on the properties, concentrations, and potential interactions (e.g., synergies) of transformation products. Most transformation products of safeners are anticipated to pose hazards to ecosystems and humans less severe than those posed by parent compounds. Nevertheless, such assumptions merit validation, particularly as dichloroacetamide safeners can become more biologically active following environmental transformations (e.g., via reductive and photolytic dechlorination).

(4) Enantioselective biotransformation of chiral safeners. Chiral safeners (e.g., benoxacor and furilazole) are added to herbicide formulations as racemates (i.e., equal mixtures of enantiomers). The potential for enantioselective biotransformation of chiral safeners remains unexplored but is expected to represent a powerful tool for source apportionment and environmental fate and transport studies. Additionally, as enantiomers of chiral herbicides frequently display disparate biological activities,[92−96] research into enantioselective biotransformation processes of chiral safeners will inform assessments of their field efficacy and ecological risks.

(5) Ecological effects of safeners and whole formulations [active ingredient(s) + safeners + additional adjuvants]. The observed toxicity of some safeners to aquatic animals, in particular, suggests a strong data need for these taxa for unexamined dichloroacetamide safeners and relevant chemical mixtures. Indeed, given their widespread use, the lack of ecotoxicity data for safeners represents a significant uncertainty in assessing ecological risks of agrochemical use.

As we have highlighted herein, although questions about the occurrence, fate, and effects of herbicide safeners remain, their mobility and transformation in the environment likely complicates assessments of their efficacy and risks. Further, given global trends toward a greater reliance on industrialized agricultural practices for crop production,[97] including herbicide use,[40] and growth in corn-based ethanol production in the United States,[98,99] environmental impacts of safeners will likely increase in coming years. Certainly, agrochemical advances will be needed to maintain crop efficiencies to sustain a surging global population. Ultimately, however, the aforementioned knowledge gaps should be addressed to ensure the sustainable development of safener-containing products that exhibit not only improved efficacy but also environmental safety.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.estlett.5b00220.

Summary of toxicity data for dichloroacetamide safeners (PDF)

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