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Specific Binding of a Dichloroacetamide Herbicide Safener in Maize at a Site That Also Binds Thiocarbamate and Chloroacetanilide Herbicides[1]

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Dichloroacetamide safeners such as *N,N*-diallyl-2,2-dichloroacetamide and (R,S)-3-dichloroacetyl-2,2,5-trimethyl-1,3-oxazolidine protect maize (*Zea mays*) against injury from thiocarbamate and chloroacetanilide herbicides. Binding activity of tritium-labeled (R,S)-3-dichloroacetyl-2,2,5-trimethyl-1,3-oxazolidine (15 Ci/mmol; referred to as [^{3}H]Saf) was characterized in extracts of etiolated maize seedlings. The binding is saturable, involves a single class of binding sites (K_d 0.12 μM; maximal binding in coleoptiles 0.53 nmol/g fresh weight, equivalent to 55 pmol/mg protein), and is sensitive to boiling and protease treatment. Binding in etiolated maize seedlings is highest in the coleoptile and lowest in the leaves. Binding of [^{3}H]Saf also occurs in etiolated sorghum (*Sorghum bicolor*) shoots but not several other cereals. There is a good correlation between known safener effectiveness and the concentration that inhibits [^{3}H]Saf binding half-maximally among 21 dichloroacetamides and related compounds. *N,N*-Diallyl-2,2-dichloroacetamide had the lowest inhibitor concentration that reduces specific binding by 50% (IC_{50}), 0.01 μM. [^{3}H]Saf binding is inhibited by 4 chloroacetanilide herbicides with IC_{50} values of 0.07 to 0.48 μM and by 12 thiocarbamate herbicides and analogs with IC_{50} values of 0.06 to 2.3 μM. The inhibition of [^{3}H]Saf binding by alachlor and *S*-ethyl dipropylthiocarbamate is competitive.

Herbicide safeners, also known as antidotes, protect crop plants from herbicides without altering their toxicity to many weeds (Hatzios, 1983, 1989). Among the most widely used safeners are the dichloroacetamides, e.g. dichlormid (R-25788), which are particularly effective at protecting maize (*Zea mays* L.) and sorghum against thiocarbamate herbicides, e.g. EPTC (Fig. 1), and chloroacetanilide herbicides, e.g. alachlor (Fig. 1).

Substantial experimental evidence indicates that the biochemical basis of dichloroacetamide action is to stimulate GSH-mediated detoxification of herbicides (Hatzios, 1983, 1989). Dichloroacetamides increase levels of GSH and GSH precursors and induce novel GSTs that recognize and detoxify herbicides (Lay et al., 1975; Lay and Casida, 1976; Adams et al., 1983; Weigand et al., 1986; Fuerst, 1987; Gronwald et al., 1987; Farago et al., 1993; Fuerst et al., 1993). The mechanism by which safeners do this is currently unknown. Based on the close structural similarity of some herbicides and safeners, it has also been proposed that safeners might act by competing with herbicides for the same site of action (Stephenson and Chang, 1978; Stephenson et al., 1979). In this model, safeners would be inactive analogs (antagonists) of herbicides; once bound, herbicides but not safeners would be able to induce the chain of events leading to toxicity. There are several reports of in vitro antagonistic effects of safeners against thiocarbamates and chloroacetanilides (Wilkinson and Smith, 1975; Wilkinson, 1981) that could be explained by competitive inhibition. Understanding the mode of action of safeners is currently limited by our incomplete knowledge of the mode of action of the herbicides themselves (Fuerst, 1987).

R-29148, here abbreviated Saf, is a potent safener of the dichloroacetamide class (Fig. 1). Saf was synthesized in a tritiated form (Latli and Casida, 1995) to study its mode of action. Here we report the identification of a proteinaceous component in etiolated maize seedlings that binds [^{3}H]Saf with high affinity. This site also binds thiocarbamate and chloroacetanilide herbicides.

MATERIALS AND METHODS

Chemicals

Racemic R-29148 and [^{3}H]Saf (specific activity 15 Ci/mmol) were prepared according to the method of Latli and Casida (1995). Dichloroacetamide safeners, chloroacetanilide herbicides, and related compounds were available from previous studies in this laboratory (Lay et al., 1975; Lay and Casida, 1976). GA_3, 2,4-D, nucleotide cofactors, GSH, and buffer salts were from Sigma, and 1,1-dichloroacetone was from Aldrich. Compounds indicated by com-

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Abbreviations: B_{max}, maximal binding; dichlormid, *N,N*-diallyl-2,2-dichloroacetamide; EPTC, *S*-ethyl dipropylthiocarbamate; GST, GSH *S*-transferase; IC_{50}, inhibitor concentration that reduces specific binding by 50%; Saf, the dichloroacetamide safener (R,S)-3-dichloroacetyl-2,2,5-trimethyloxazolidine, also known as R-29148; [^{3}H]Saf, the radioligand form of R-29148; SafBA, safener binding activity.

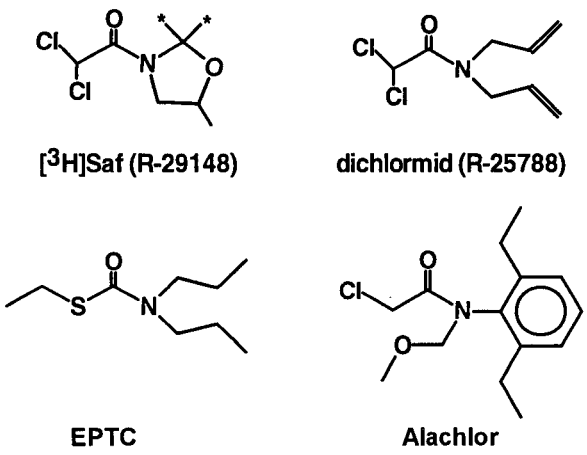

Figure 1. Structures of [^{3}H]Saf and selected compounds discussed in the text. R-29148 and dichlormid are dichloroacetamide safeners, EPTC is a thiocarbamate herbicide, and alachlor is a chloroacetanilide herbicide.

mon names were defined chemically by Tomlin (1994) and Hatzios (1989).

Plant Material

Maize (*Zea mays* L. inbred B73) was grown in the dark for 4 to 7 d on wet paper towels in 15- × 20-cm covered plastic boxes in a laboratory cupboard. For most experiments, the entire shoot above the seed was used. Seedlings were ground in a mortar and pestle in 50 mM Tris-HCl, pH 8, 0.4 M Suc, and 20 μg/mL PMSF. The homogenate was filtered through two layers of cheesecloth, and the debris was reground and refiltered. The final buffer to tissue ratio was 5:1 (200 mg fresh weight/mL). Subsequent dilutions of the plant extract were with 50 mM Tris-HCl, pH 8.

Oats (*Avena sativa* cv Garry), barley (*Hordeum vulgare* cv CM72), wheat (*Triticum aestivum* cv Yecora Rojo), sorghum (*Sorghum vulgare* cv P-954063), beans (*Phaseolus vulgaris* cv Blue Lake 274), cucumber (*Cucumis sativus* cv Straight 8), and cabbage (*Brassica oleracea* cv early Jersey Wakefield) were grown similarly, and the entire shoot was harvested.

Binding Assay

Crude plant extract (final volume 1.0 mL) was added to 1.5-mL microfuge tubes containing 12 nM [^{3}H]Saf (final concentration; approximately 400,000 dpm). To measure nonspecific binding, unlabeled Saf was added to the tubes to a final concentration of 3 μg/mL (13 μM) prior to addition of the plant extract. The tubes were incubated at 21°C for 1 h and then filtered using a multiwell vacuum filtration manifold through GF/A filters that had been soaked for at least 1 h in 0.3% polyethylenimine (Bruns et al., 1983). The filters were quickly washed twice with 5 mL of water and placed in vials in scintillation cocktail and counted after at least 24 h. All results were corrected for quench and are expressed as dpm. Protein was measured by the method of Bradford (1976) with BSA as standard.

Measurement of Inhibitor Potency for [^{3}H]Saf-Binding Activity

Test compounds were always added to the microfuge tubes along with [^{3}H]Saf prior to addition of plant extract. Each compound was tested with at least five concentrations, in duplicate, spanning the region that inhibited specific [^{3}H]Saf binding by 50% (IC_{50}). Most test compounds were made as stock solutions in absolute ethanol; since binding was strongly inhibited by 1% ethanol, final ethanol concentrations were kept to 0.1% or less.

Test of Inducibility of [^{3}H]Saf-Binding Activity

Roots from 4-d-old maize seedlings germinated on wet paper towels were excised and placed in 100-mm glass Petri plates containing a piece of Whatman No. 1 filter paper, 5 mL of water, and the appropriate test compounds. The roots (typically 15 per plate) were incubated with 13 μM Saf or 11 μM alachlor in the dark for 48 h. For measurement of GSH levels, the roots were ground in 70% (v/v) ethanol and centrifuged (5000*g*, 10 min). Total ethanol-soluble thiols (taken to be predominantly GSH) were measured in the supernatant using 5,5′-dithiobis(2-nitrobenzoic acid) (Ellman's reagent) (Lay and Casida, 1976). For measurement of binding, roots were treated identically and ground in buffer as described above for the standard binding assay. The extracts were then passed through a prepacked desalting column (Pharmacia PD-10) to remove the Saf and alachlor that had been taken up by the roots. The extracts were then assayed for [^{3}H]Saf binding and protein as described above.

Binding in Mouse Tissues

Mouse (male Swiss-Webster) tissues (liver, muscle, brain, stomach, heart, kidney, spleen, lung, and blood) were ground in 0.1 M potassium phosphate, pH 7.4, at a buffer to tissue ratio of 4:1. [^{3}H]Saf binding in the tissue extracts was assayed with at least three tissue concentrations ranging from 5 to 150 mg fresh weight/mL.

RESULTS

Specific Binding of [^{3}H]Saf to Maize Shoot Extracts

Cell-free extracts of total etiolated maize shoots show specific binding of [^{3}H]Saf. The level of specific but not nonspecific binding is strongly affected by the concentration of plant extract. Above 100 mg fresh weight/mL, specific binding is difficult or impossible to detect (Fig. 2). The optimum concentration for whole shoot extracts is approximately 40 mg fresh weight/mL (Fig. 2). Nonspecific binding is independent of plant extract concentration (Fig. 2). Specific binding is approximately the same at pH 7 in Mops buffer, pH 8 in Tris buffer, or pH 9 in Tris buffer but is about 75% less at pH 6 in Mes buffer. Suc (0.4 M) promotes binding when present in the extraction buffer but inhibits by approximately 30% when present in the binding assay. Binding is undetectable if the glass fiber filters are not pretreated with polyethylenimine.

Binding activity is completely destroyed by boiling the plant extracts for 5 min or treating them with chymotrypsin or proteinase K (2 mg/mL, 2 h, 37°C), indicating that the [^{3}H]Saf-binding activity is due to a protein. The SafBA is soluble, since 100% of the binding activity remains in the supernatant after ultracentrifugation (100,000*g*, 20 min) of the maize extract.

[^{3}H]Saf binding is detected in all parts of maize seedlings but is highest in the coleoptile and lowest in the leaves (Fig. 3). More than half of the binding activity is in the coleoptile when expressed on either a protein or a total shoot basis (Table I).

Kinetics of [^{3}H]Saf Binding

At room temperature (21°C), binding reaches equilibrium after 40 min and is stable for at least an additional 100 min (Fig. 4). The binding that occurs after 60 min is fully reversible by 13 μM unlabeled Saf (Fig. 4). A Scatchard plot indicates a single class of binding sites (K_d 0.12 μM; B_{max} 0.53 nmol/g fresh weight) in coleoptiles (Fig. 5). Bound [^{3}H]Saf was desorbed from the glass fiber filters by soaking them in water for 24 h. When analyzed by HPLC (Latli and Casida, 1995), all of the radioactivity co-eluted with standard Saf, suggesting that binding is not accompanied by chemical modification of [^{3}H]Saf.

Potential Endogenous Modulators of [^{3}H]Saf Binding

GSH (5 mM) does not affect [^{3}H]Saf binding, although DTT inhibits binding half-maximally at 1.5 mM. β-Nicotinamide adenine coenzymes inhibit [^{3}H]Saf binding with IC_{50} values of >2, 2.1, 0.7, and 0.9 mM for NAD^+, $NADP^+$, NADH, and NADPH, respectively. Neither EDTA nor Mg^{+2} affects binding (IC_{50} >5 mM).

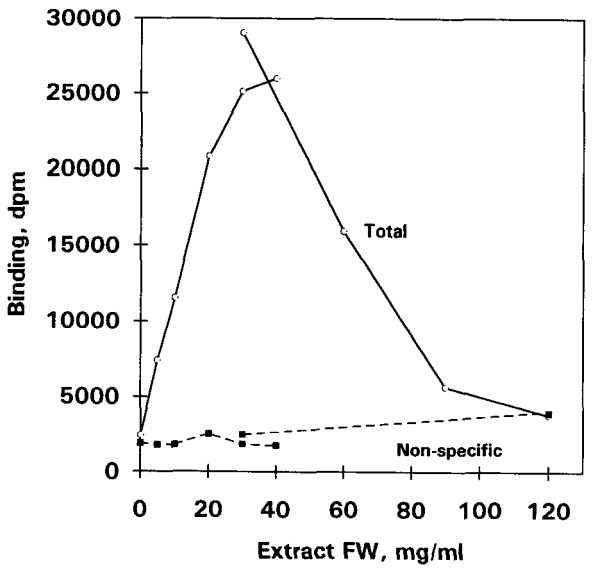

Figure 2. Effect of the concentration of maize shoot extract on [^{3}H]Saf binding. The same shoot extract was used for one experiment at low concentration and another experiment at high concentration. Total volume of the binding reaction mixture was made to 1.0 mL in every case with 50 mM Tris-HCl, pH 8.0. ○, Total binding; ■, nonspecific binding. FW, Fresh weight.

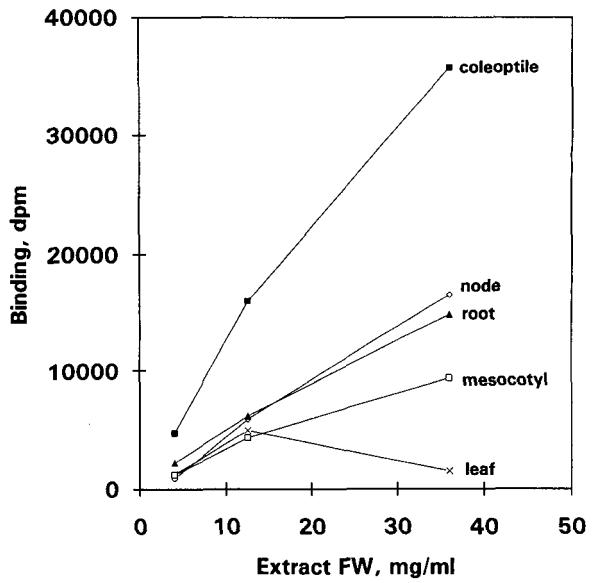

Figure 3. Specific [^{3}H]Saf binding in different tissues of etiolated maize seedlings. Component parts of 32 seedlings (5 d old) were ground in a final buffer to a fresh weight (FW) tissue ratio of 5:1.

Lack of Inducibility of SafBA by Safener or Herbicide Treatment

When excised roots are treated for 48 h with 13 μM (3 μg/mL) Saf or 11 μM (3 μg/mL) alachlor, GSH levels increase, as previously reported (Lay et al., 1975), but binding of [^{3}H]Saf is not altered (Table II).

Effect of Dichloroacetamide Safeners and Related Compounds and Other Safeners on [^{3}H]Saf Binding

Twenty dichloroacetamides and related compounds were tested for ability to compete with [^{3}H]Saf in binding assays. All compounds known to be effective safeners inhibit binding, with IC_{50} values of 0.01 to 9.4 μM (Table III). Dichlormid (1[3]) has the lowest IC_{50}, followed closely by its *N,N*-dipropyl, trichloroacetamide, and *N*-allyl,*N*-propyl analogs (2–4). Dichlormid in which the chlorine atoms are replaced by methyl groups (5) also has high affinity, even though it is inactive as a safener. The monochloro analog of dichlormid, *N,N*-diallyl-2-chloroacetamide (9), which is both a herbicide and a moderate safener, is an effective inhibitor of [^{3}H]Saf binding. The single serious discrepancy among the compounds tested is nitrile 19, which is rated as having "moderate" safener activity (Lay and Casida, 1976) yet does not inhibit binding even at 29 μM. Safeners that are chemically unrelated to R-29148 (22–28) do not inhibit binding.

[3] Numbers in parentheses correspond to structures shown in Tables III and IV.

Table I. *Distribution of [^{3}H]Saf binding in maize seedlings*

The values shown are for a final tissue concentration of 12 mg fresh weight/mL (see Fig. 3). Numbers in parentheses are percentages of total binding.

Tissue	Protein Basis	Fresh Wt Basis	Whole Plant Basis
	pmol/mg	*pmol/g*	*pmol/seedling*
Coleoptile	6.85 (55.1)	40.0 (42.7)	1124 (54.8)
Leaf	0.44 (3.5)	12.5 (13.3)	66.4 (3.2)
Node[a]	0.83 (6.7)	14.8 (15.8)	148 (7.2)
Mesocotyl	1.77 (14.2)	10.9 (11.6)	322 (15.7)
Root	2.54 (20.4)	15.5 (16.5)	392 (19.1)

[a] Node included approximately 1.5 mm of tissue on either side.

Effect of Herbicides on [^{3}H]Saf Binding

Chloroacetanilide and thiocarbamate herbicides are also very effective inhibitors of [^{3}H]Saf binding, particularly metolachlor (29), alachlor (30), and EPTC (34), which have IC_{50} values of 0.04 to 0.11 μM (Table IV). Double-reciprocal plots with alachlor and EPTC indicate that both compounds compete with [^{3}H]Saf in a competitive manner (Fig. 6). The chemically unrelated herbicides atrazine (45) and 2,4-D (47) do not inhibit binding, nor do GA_3 (46) and 2,6-dichloroisonicotinic acid (48).

Distribution of [^{3}H]Saf Binding Activity in Other Plants and in Mouse Tissues

[^{3}H]Saf binding comparable to that found in maize was found in extracts of shoots (1.43 pmol/mg protein) and roots (0.91 pmol/mg protein) of sorghum. Specific binding in extracts of 4- to 7-d-old shoots of three other species in the Poaceae family (wheat, barley, and oat) and in three dicotyledonous plants (cucumber, bean, and cabbage) was less than 0.1 pmol/mg protein. In all cases, a range (5–160 mg fresh weight/mL) of extract concentrations was tested against the possibility that binding in other plants, like maize, is detectable only in a limited range of concentrations. Binding in roots of species other than maize and sorghum was not tested.

No in vitro specific binding was detectable in mouse at any concentration of tissue tested.

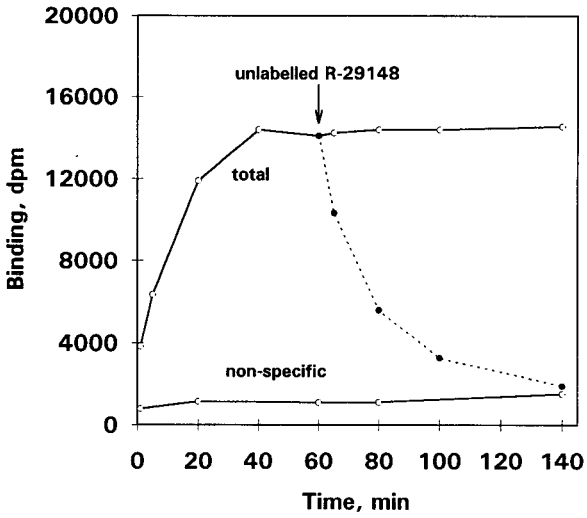

Figure 4. Time course of binding of [^{3}H]Saf to cell-free extracts of etiolated maize shoots. Extract concentration was 40 mg fresh weight/mL. O——O (top), Total binding; ●- - - -●, total binding after addition of 15 μM unlabeled Saf at 60 min; O——O (bottom), nonspecific binding.

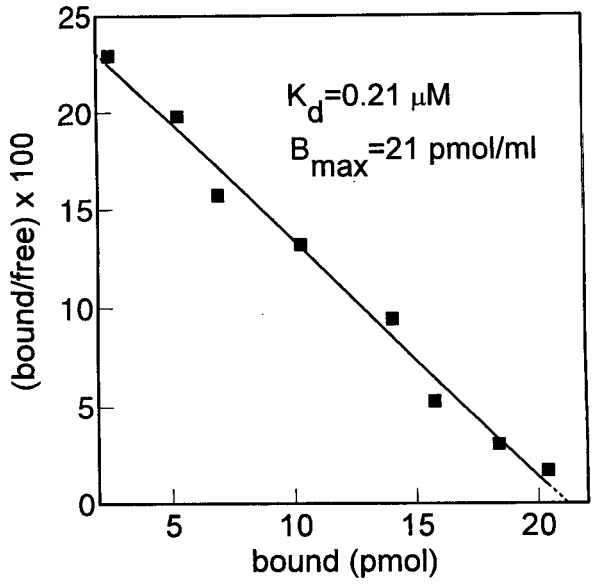

Figure 5. Scatchard plot of [^{3}H]Saf binding in cell-free extracts of etiolated maize coleoptiles. Assays were done at an extract concentration of 40 mg fresh weight/mL, equivalent to 385 μg protein/mL. Calculated receptor concentration is equivalent to 530 pmol/g fresh weight or 54.8 pmol/mg protein.

DISCUSSION

Etiolated maize seedlings contain a single class of sites that bind [^{3}H]Saf with high affinity in a saturable and reversible manner. SafBA is present in all tissues of the etiolated maize seedling but is especially abundant in the coleoptile. Several studies implicate the coleoptile as a particularly critical tissue for response both to dichloroacetamide safeners and to thiocarbamate and chloroacetanilide herbicides (Hickey and Krueger, 1974; Wilkinson, 1982; Fuerst, 1987; Fuerst et al., 1991). However, it is possible that the tissue distribution results do not accurately

Table II. *Test of inducibility of [^{3}H]Saf binding by pretreatment with Saf or alachlor*

Excised roots (15 per treatment, in duplicate) were treated with 13 μM Saf or 11 μM alachlor for 48 h. The roots were ground and extracted as described in "Materials and Methods," passed through a desalting column, and assayed for [^{3}H]Saf binding and protein. GSH levels were measured in a separate set of roots, in duplicate.

Treatment	GSH	Specific [^{3}H]Saf Binding	
	% of control	*pmol/g fresh wt*	*pmol/mg protein*
None	100	8.87	1.65
Saf	190	7.36	1.67
Alachlor	167	8.37	1.70

Table III. *Potency of dichloroacetamide safeners, related compounds, and other types of safeners as inhibitors of [^{3}H]Saf binding in extracts of etiolated maize shoots (40 mg fresh weight/mL)*

All compounds were tested in duplicate with at least five concentrations spanning the IC_{50}.

No.[a]	Structure or Name[b]	IC_{50}
		μM
	Dichloroacetamide safeners and related compounds	
1	Dichlormid (R-25788) (see Fig. 1)	0.010
2	$Cl_2CHC(O)N(CH_2CH_2CH_3)_2$	0.013
3	$Cl_3CC(O)N(CH_2CH{=}CH_2)_2$	0.014
4	$Cl_2CHC(O)N(CH_2CH{=}CH_2)C_3H_7$	0.014
5	$(CH_3)_2CHC(O)N(CH_2CH{=}CH_2)_2$	0.016
6	$Br_3CC(O)NHC(CH_3)_2C{\equiv}CH$	0.090
7	Saf (see Fig. 1)	0.12
8	5-demethyl Saf (R-28725; AD-2)	0.16
9	$ClCH_2C(O)N(CH_2CH{=}CH_2)_2$ (CDAA)	0.31
10	Benoxacor (CGA 154281)[b]	0.74
11	$Cl_2CHC(O)NHC(CH_3)_3$	2.3
12	MG-191[b]	4.4
13	AD-67[b]	6.0
14	$Cl_2CHC(O)NHCH_2CH{=}CH_2$	7.0
15	$Cl_2CHC(O)NH$-ϕ-2-CH_2CH_3	8.0
16	Furilazole (MON 13900)[b]	9.4
17	$(CH_3)_2CHC(O)NHC(CH_3)_2C{\equiv}CH$	9.5
18	$Cl_2CHC(O)NH$-2-benzothiazole	>22[c]
19	$Cl_2CHC(O)NHC(CH_3)_2C{\equiv}N$	>29
20	$CH_3CH_2OC(O)C(O)N(CH_2CH{=}CH_2)_2$	>29
21	1,1-Dichloroacetone[d]	580
	Other safeners	
22	Oxabetrinil (CGA 92194)	>22
23	Flurazole (MON 4606)	>16
24	Chloquintocet (CGA 185072)	>1.6
25	Fenchlorazole	>14
26	Fenclorim (CGA 123407)	>10
27	Naphthalic anhydride	>17
28	Naphthalic anhydride, hydrolyzed[e]	>17

[a] Safener effectiveness (Lay and Casida, 1976; Stephenson and Chang, 1978): superior, 1, 7, and 8; superior to moderate, 2; good, 4, 11, and 15; moderate, 3, 6, 9, 14, and 19; little or no, 5, 17, 18, and 20. Comparable data are unavailable for 10, 12, 13, and 16, but all four are being used or have been used commercially as safeners. [b] 10 (Benoxacor) is (*R,S*)-4-dichloroacetyl-3,4-dihydro-3-methyl-2*H*-1,4-benzoxazine); 12 (MG-191) is 2-dichloromethyl-2-methyl-dioxolane; 13 (AD-67) is *N*-dichloroacetyl-1-oxa-4-aza-spiro-4,5-decane; 16 (furilazole) is (*R,S*)-3-dichloroacetyl-2,2-dimethyl-5-furan-1,3-oxazolidine. [c] The "greater than" symbol indicates that the stated concentration inhibited [^{3}H]Saf binding by less than 50%. [d] Included as the putative hydrolysis product of MG-191 (12). [e] Naphthalic anhydride was hydrolyzed by treatment with 0.1 M NH_4HCO_3 for 1 h.

reflect the distribution of SafBA, since at high tissue levels [^{3}H]Saf binding is not proportional to the tissue concentration, due either to endogenous inhibitor(s) or to differential binding of maize proteins to polyethylenimine-treated filters.

There is a good correlation between inhibition of [^{3}H]Saf binding and safener effectiveness among dichloroacetamides and related compounds. Possible exceptions to this relationship are dichloroacetamide 19 and the dichlormid isoster (5) in which the chlorines are replaced by methyls, which may be metabolized too fast to be effective.

[^{3}H]Saf binding is also inhibited by low concentrations of chloroacetanilide and thiocarbamate herbicides in a competitive manner. This observation could support the hypothesis that safeners are receptor antagonists of herbicides (Stephenson and Chang, 1978; Stephenson et al., 1979). Dichlormid is used commercially with EPTC and butylate at a ratio of 1:11 and 1:24, respectively, which is in reasonable agreement with the ratios of their IC_{50} values (1:11 and 1:54, respectively). On the other hand, Saf is used commercially with EPTC and butylate at ratios of 1:24 and 1:50 but the ratios of their IC_{50} values are 0.9 and 4.5, respectively (Weed Science Society of America, 1989). In any case, correlation of field application rates and binding affinities is difficult, since the actual relative concentrations of a herbicide and its safener at their site(s) of action are dependent on their relative rates of uptake, translocation, and metabolism.

Dichloroacetamides are proposed to be metabolized and to act by mechanisms that involve both GSH/GST and Cyt P450s. Thus, dichlormid is converted in plants via hydroxylation and dechlorination to an oxamic acid, which is also formed in rat liver preparations via both GSH/GST and P450 pathways (Miaullis et al., 1978). The dichloroacetamide antibiotic chloramphenicol is also converted to an oxamic acid and covalently binds to P450 in the process (Pohl and Krishna, 1978). These relationships raise the question of whether SafBA is due to a GST or P450. SafBA is unlikely to be attributed to a GST for three reasons: it is not induced by either R-29148 or alachlor, [^{3}H]Saf released

Table IV. *Potency of chloroacetanilide and thiocarbamate herbicides and other compounds as inhibitors of [^{3}H]Saf binding in extracts of etiolated maize seedlings*

Experimental conditions are the same as in Table III.

No.	Structure or Name	IC_{50}
		μM
	Chloroacetanilide herbicides	
29	Metolachlor	0.040
30	Alachlor (see Fig. 1)	0.070
31	Acetochlor	0.18
32	Propachlor	0.48
	Thiocarbamate herbicides and related compounds	
33	$CH_3SC(O)N(CH_2CH_2CH_3)_2$	0.061
34	EPTC (see Fig. 1)	0.11
35	Vernolate	0.12
36	$CH_3SC(O)N(CH_2CH_3)_2$	0.16
37	Pebulate	0.23
38	Molinate	0.38
39	$CH_3CH_2S(O)C(O)N(CH_2CH_2CH_3)_2$ (EPTC sulfoxide)	0.53
40	Butylate	0.54
41	Triallate	1.2
42	Cycloate	1.6
43	Thiobencarb	2.3
44	$CH_3CH_2SC(O)NHCH_2CH_2CH_3$	19
	Other compounds	
45	Atrazine	>25
46	GA_3	>15
47	2,4-D	>24
48	2,6-Dichloroisonicotinic acid	>16

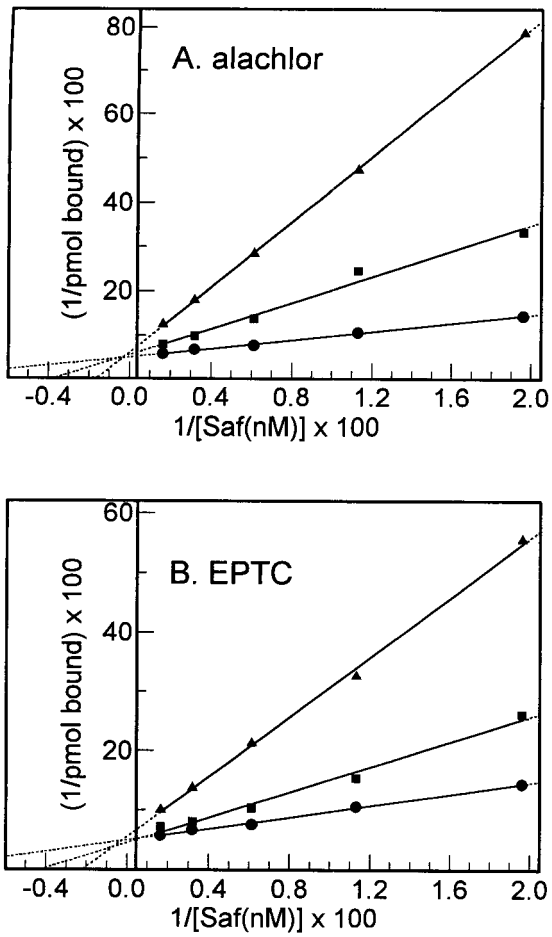

Figure 6. Double-reciprocal plots of the inhibition of specific [^{3}H]Saf binding by alachlor or EPTC. A, Alachlor. ●, [^{3}H]Saf alone; ■, [^{3}H]Saf plus 0.12 μM alachlor; ▲, [^{3}H]Saf plus 0.36 μM alachlor. B, EPTC. ●, [^{3}H]Saf alone; ■, [^{3}H]Saf plus 0.12 μM EPTC; ▲, [^{3}H]Saf plus 0.36 μM EPTC.

from its binding site has the same HPLC retention time as Saf, and binding is not affected by GSH in vitro. SafBA is probably not a Cyt P450, since most P450s are integral membrane proteins, whereas SafBA is soluble.

Dichloroacetamide safeners are also widely used on sorghum (Hatzios, 1983), which also has SafBA. Oats, which do not respond to dichloroacetamide safeners (Lay et al., 1975), do not have detectable SafBA. Dichloroacetamide safeners are somewhat effective against thiocarbamates and chloroacetanilides in plants other than maize and sorghum, e.g. barley, bean, and wheat (Stephenson and Chang, 1978; Hatzios, 1983), which do not have SafBA. Therefore, if SafBA is involved in the action of dichloroacetamides in these species, either the maize assay for SafBA is not appropriate or safening action occurs through a different pathway in other plants. Furthermore, it appears that other classes of safeners, which do not compete for [^{3}H]Saf binding, must also act through a different pathway. The two hypotheses of safener action, stimulation of detoxification and competition for the same site, are not mutually exclusive (Fuerst, 1987). For example, herbicides themselves can protect plants against subsequent exposure to the same or related herbicides (Ezra et al., 1985), and herbicides elevate GSH and GSH precursor levels and induce GSTs (Dean et al., 1990; Farago and Brunold, 1990; Jablonkai and Hatzios, 1991). A model consistent with existing experimental evidence and with the hypothesis that SafBA is the initial site of action of Saf is that SafBA is involved in transduction of two pathways: one leading to elevation of GSH and induction of GSTs, which is triggered by all three classes of compounds (dichloroacetamide safeners and thiocarbamate and chloroacetanilide herbicides), and the other leading to phytotoxicity, which is triggered only by the herbicides.

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