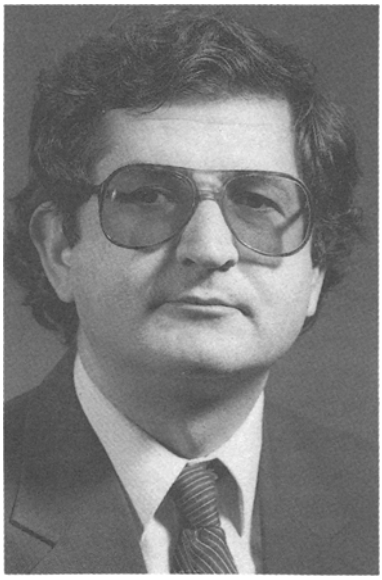

Kriton Kleanthis Hatzios, born 1949, Director, Virginia Agricultural Experiment Station (VAES) and Assoc. Dean for Research, College of Agriculture and Life Sciences, Virginia Polytechnic Inst. and State U. (Virginia Tech). B.Sc. in Agricultural Sci./Chem. (1972), Aristotelian U. of Thessaloniki, Greece; M.Sc. (1977) and Ph.D. (1979), both at Michigan State U.; 1979, Asst. Prof.; 1984, Assoc. Prof.; 1988, Prof., all at Virginia Tech. 1997–2000, Head of the Dept. of Plant Pathology, Physiology and Weed Science at Virginia Tech. 1999– , Director, VAES and Assoc. Dean for Research, College of Agric. and Life Sciences, Virginia Tech. Dr. Hatzios served as tutor (major advisor, co-advisor or lab res. supervisor) to 22 doctoral and three master's candidates. *Main research interests:* Action, fate and selectivity of herbicides and herbicide safeners; herbicide resistance in crops and weeds. *Publications:* Four books authored or edited: *Metabolism of Herbicides in Higher Plants* [co-author with D. Penner, 1982, Burgess Publishing Company, Minneapolis, MN]; *Crop Safeners for Herbicides* [co-editor with R. E. Hoagland, 1989, Academic Press, San Diego, CA]; *Regulation of Enzymatic Systems Detoxifying Xenobiotics in Plants* [editor, 1997, Kluwer Publishing Co., Dordrecht, the Netherlands]; and *Herbicide Handbook – 7th ed. Supplement* [editor, 1998, Allen Press, Lawrence, KS]; 115 refereed journal papers, 24 book chapters, eight reviewed proceeding papers, 200 abstracts. *Editorial duties:* 1991 – present, member of the Editorial Board of *Pesticide Biochemistry and Physiology;* 1986 – 89, Assoc. Editor of *Weed Science. Awards and honors:* Fellow Award by the American Association for the Advancement of Science (AAAS, 2001); the Outstanding Young Scientist Award (1986), the Outstanding Research Award (1994), the Fellow Award (1995), the Outstanding Teacher Award (2001), all by Weed Science Society of America; the Outstanding Young Weed Scientist Award (1987), the Scientist of the Year Award (1997), both conferred by the Southern Weed Science Society.

Herbicide Safeners: Effective Inducers of Plant Defense Gene-Enzyme Systems

Safeners (also known as antidotes) are synthetic chemicals used worldwide to protect crop plants from herbicide injury without reducing weed control (2,5,7,12). Marketed safeners are members of chemically diverse groups including dichloroacetamides (*e.g.* dichlormid, benoxacor, furilazole and R-29148), naphthopyranones (*e.g.* naphthalic anhydride), dichloromethyl acetals and ketals (*e.g.* MG-191), oxime ether derivatives (*e.g.* fluxofenim), 2,4-disubstituted thiazolecarboxylates (*e.g.* flurazole), phenylpyrimidines (*e.g.* fenclorim), phenyl pyrazoles (*e.g.* fenchlorazole-ethyl and mefenpyr-diethyl), quinolinoxycarboxylic acid esters (*e.g.* cloquintocet-mexyl), thiocarbamates (*e.g.* dimepiperate), methylbenzyl-tolylureas (*e.g.* daimuron) and benzenesulfonamides (*e.g.* 2-CBSU) (2,5,7,12). The chemical structures of some commonly used safeners are shown in Figure 1.

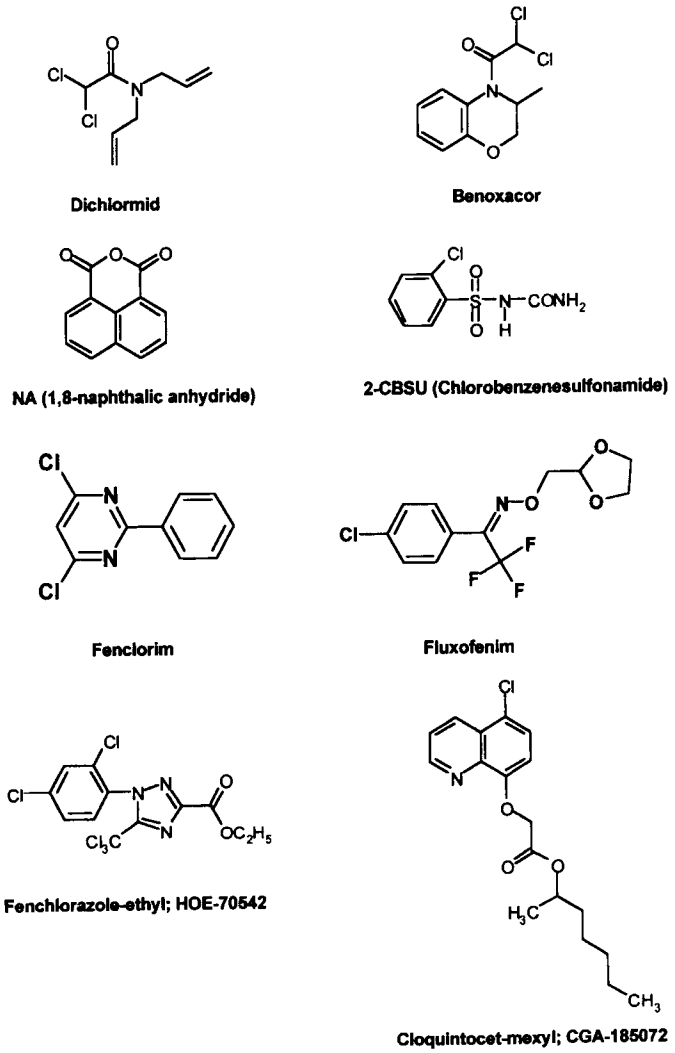

Fig. 1. Chemical structures of selected safeners. Dichlormid, benoxacor, NA and 2-CBSU are maize safeners; fenclorim is a rice safener; fluxofenim is a sorghum safener; fenchlorazole-ethyl and cloquintocet-mexyl are wheat safeners.

Extensive research on the interactions of safeners with their respective herbicides over the past 30 years has demonstrated that: *(a)* safeners exhibit a high degree of botanical and chemical specificity; *(b)* protected grass crops are moderately tolerant to the antagonized herbicides; and *(c)* safeners are most effective when applied prior to or simultaneously with the herbicides whose injury they prevent (2,5,7,12).

In the field, safeners protect large-seeded grass crops such as maize, grain sorghum, wet-sown rice, and wheat against thiocarbamate, chloroacetanilide, aryloxyphenoxypropionate, sulfonylurea, imidazolinone, triketone, and isoxazole herbicides. Safeners are

applied either to the crop seed prior to planting (*seed safeners*) or to the soil or crop together with the herbicides in a single formulation package. The safener-to-herbicide doses in these mixtures range from 1:6 to 1:30 (2,5,7).

Safener-induced enhancement of herbicide detoxification in safened plants is widely accepted as the major mechanism of safener action (2,5,7,12). Most safeners resemble structurally their respective herbicides and they induce cofactors such as glutathione (GSH) and herbicide-detoxifying enzymes such as glutathione *S*-transferases (GST), cytochrome P450 mono-oxygenases (P450), and glucosyl transferases (GT). In addition, safeners can stimulate the vacuolar transport of glutathione or glucose conjugates of selected herbicides (2,5,10,12).

The induction of P450 and GST transcripts by safeners appears to be tissue-specific and developmentally regulated (1,2,5,6,10,11,12). Safeners induce the activity of numerous plant P450s catalyzing aryl hydroxylation, ring-methyl hydroxylation, and *N*-demethylation reactions involved in the metabolic detoxification of chloroacetanilide, aryloxyphenoxypropionate, sulfonylurea, imidazolinone, and sulfonamide herbicides in protected grass crops (2,5,11,12).

A strong correlation between the ability of a safener to increase GST activity and its efficacy in protecting maize, grain sorghum, rice and wheat from herbicide injury has been demonstrated (1,6,10). Pretreatment of maize, rice and grain sorghum with safeners such as dichlormid, benoxacor, fenclorim and flurazole enhances greatly the crops' low intrinsic tolerance to thiocarbamate and chloroacetanilide herbicides by inducing GST activity, and in turn elevating the rate of the herbicide detoxification *via* GSH conjugation (1,2,5,6,10,12). Safeners may also elevate GSH levels in protected plants either directly by regulating the assimilatory sulfate reduction to cysteine and activating key enzymes involved in the biosynthesis of GSH, or indirectly by inducing the activity of glutathione reductase (2,5,6).

Multiple GST isozymes conjugating herbicides have been characterized in maize, wheat, sorghum, and rice (1,6,10). Some of these GST isozymes are constitutive and some are inducible by safeners. Several safener-induced cDNA clones coding for GSTs (*e.g. Gst27*) have been isolated from maize and other crops (1,6,10). Hershey and Stoner (8) developed a novel gene expression system, regulated by a safener and a promoter derived from a safener-responsive gene. They isolated two cDNA clones, designated as *In2-1*, *In2-2*, whose mRNAs were induced rapidly in root and leaf tissues of maize after treatment with the benzenesulfonamide safener 2-CBSU. In subsequent studies, De Veylder *et al.* (3) transformed *Arabidopsis thaliana* with a reporter gene construct under the control of the *In2-2* promoter, which is activated by the 2-CBSU safener. It was found that the *In2-2* promoter activity could be induced by several safeners and the herbicide chlorsulfuron. This indicates that there may be a correlation between *In2-2* expression and inhibition of acetolatate synthase activity by sulfonylurea herbicides (3).

Plants respond to biotic or abiotic stresses by a sequence of events including stress recognition, signal transduction and defense gene activation (4,10). The involvement of a common factor in the signaling transduction pathway, from the initial recognition of the stimulus to the activation of gene expression of plant *Gst* genes, is very likely. This is supported by the fact that *Gst* genes are activated by oxidative stress (H_2O_2) and the activity of the encoded proteins is needed for cellular protection against oxidative damage (1,6,10). Treatment of plants with herbicides and safeners constitutes a chemical stress, and could

act as an elicitor of the signal transduction systems present in plants. Indeed, apart from safeners, many biotic or abiotic stresses (*e.g.* pathogen attack, wounding, auxins, ethylene, abscisic acid, heat shock, heavy metals, and environmental conditions generating oxidative stress) act also as activators of genes coding for plant defense enzymes (1,2,6,10).

Signal transduction complexes in plants may be permanent or ephemeral and are characterized by a strong spatial dimension, which is exemplified by the presence of membrane-bound receptors, channels, G proteins and kinases found in plant cells (14). After ligand binding and autophosphorylation, receptor kinases may act as nucleation sites for the construction of ephemeral signaling complexes that may contain many proteins and facilitate the regulation of specific transcription factors (14). Protein-protein interactions are very important in signal transduction regardless of whether the proteins are membrane-bound or sitting on promoter regions (14).

Walton and Casida (15) discovered a membrane-bound protein with high affinity for binding of dichloroacetamide safeners and thiocarbamate and chloroacetanilide herbicides in maize and designated it as safener-binding protein (SafBP). A clear qualitative correlation was observed between safener potency and specific binding to the SafBP, which is most abundant in the coleoptile and scarcest in the leaves of maize (15). Subsequent studies with purified SafBP by Scott-Craig *et al.* (13) showed that the sequence of SafBP had significant homology to a class of plant *O*-methyltransferase enzymes. It was concluded that SafBP might not be a functional receptor site involved in the action of the dichloroacetamide safeners and/or herbicides (13).

Multiple regulatory elements are present in the promoters of genes coding for specific defense enzymes (*e.g.* GST and P450), some of which react to specific signals and others which react to more general stress-related signals (10). However, in contrast to mammalian systems, where xenobiotic regulating elements (XRE) are found in multiple copies of GST and P-450 genes, plant GST promoters do not contain functional XRE (10). Instead, some plant GST genes are known to possess octopine synthase (*ocs)* elements within their promoter regions (10). The *ocs* elements of plant GST promoters appear to be stress-inducible elements, responding to biotic and abiotic agents such as plant hormones, heavy metals, pathogen attack, wounding and environmental conditions generating oxidative stress (10). Jepson *et al.* (9) have proposed the presence of a safener response element in the promoters of *Gst* genes coding for maize GSTs.

In spite of their costly development and the increased commercialization of transgenic crops resistant to herbicides, herbicide safeners remain a viable tool for optimizing the selective use of current and new herbicides. Continuing progress in microarray technology, proteomics and metabolomics will advance our understanding of the mechanisms involved in stress recognition, signal transduction and activation of defense genes in plants. In turn, such advances will elucidate more clearly how safeners work at the molecular level in protecting crop plants against herbicide injury.

Kriton K. Hatzios
Dept. of Plant Pathology, Physiology, and Weed Science
Virginia Polytechnic Institute and State University
Blacksburg, VA 24061-0402, USA
[Fax: +1-540-231-4163; e-mail: hatzios@vt.edu]

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