Summary of Herbicide Mechanism of Action According to the Weed Science Society of America (WSSA)

1

Acetyl CoA Carboxylase (ACCase) Inhibitors

Aryloxyphenoxypropionate (FOPs) cyclohexanedione (DIMs) and phenylpyrazolin (DENs) herbicides inhibit the enzyme acetyl-CoA carboxylase (ACCase), the enzyme catalyzing the first committed step in *de novo* fatty acid synthesis (Burton 1989; Focke and Lichtenthaler 1987). Inhibition of fatty acid synthesis presumably blocks the production of phospholipids used in building new membranes required for cell growth. Broadleaf species are naturally resistant to cyclohexanedione and aryloxyphenoxy propionate herbicides because of an insensitive ACCase enzyme. Similarly, natural tolerance of some grasses appears to be due to a less sensitive ACCase (Stoltenberg 1989). An alternative mechanism of action has been proposed involving destruction of the electrochemical potential of the cell membrane, but the contribution of this hypothesis remains in question.

2

Acetolactate Synthase (ALS) or
Acetohydroxy Acid Synthase (AHAS) Inhibitors

Imidazolinones, pyrimidinylthiobenzoates, sulfonylaminocarbonyltriazolinones, sulfonylureas, and triazolopyrimidines are herbicides that inhibit acetolactate synthase (ALS), also called acetohydroxyacid synthase (AHAS), a key enzyme in the biosynthesis of the branched-chain amino acids isoleucine, leucine, and valine (LaRossa and Schloss 1984). Plant death results from events occurring in response to ALS inhibition and low branched-chain amino acid production, but the actual sequence of phytotoxic processes is unclear.

3 **15** **23**

Mitosis Inhibitors

Benzamide, benzoic acid (DCPA), dinitroaniline, phosphoramidate, and pyridine herbicides (Group 3) are examples of herbicides that bind to tubulin, the major microtubule protein. The herbicide-tubulin complex inhibits polymerization of microtubules at the assembly end of the protein-based microtubule but has no effect on depolymerization of the tubule on the other end (Vaughn and Lehnen 1991), leading to a loss of microtubule structure and function. As a result, the spindle apparatus is absent, thus preventing the alignment and separation of chromosomes during mitosis. In addition, the cell plate can not be formed. Microtubules also function in cell wall formation. Herbicide-induced microtubule loss may cause the observed swelling of root tips as cells in this region neither divide nor elongate.

The carbamate herbicides, carbetamide, chlorpropham, and propham (23), are examples of herbicides that inhibit cell division and microtubule organization and polymerization.

Acetamide, chloroacetamide, oxyacetamide, and tetrazolinone herbicides (Group 15) are examples of herbicides that are currently thought to inhibit very long chain fatty acid (VLCFA) synthesis (Husted et al. 1966; Böger et al. 2000). These compounds typically affect susceptible weeds before emergence, but do not inhibit seed germination.

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Synthetic Auxins

Benzoic acids, phenoxycarboxylic acids, pyridine carboxylic acids, and quinoline carboxylic acids (Group 4) are herbicides that act similar to that of endogenous auxin (IAA) although the true mechanism is not well understood. The specific cellular or molecular binding site relevant to the action of IAA and the auxin-mimicking herbicides has not been identified. Nevertheless, the primary action of these compounds appears to affect cell wall plasticity and nucleic acid metabolism. These compounds are thought to acidify the cell wall by stimulating the activity of a membrane-bound ATPase proton pump. The reduction in apoplasmic pH induces cell elongation by increasing the activity of enzymes responsible for cell wall loosening. Low concentrations of auxin-mimicking herbicides also stimulate RNA polymerase, resulting in subsequent increases in RNA, DNA, and protein biosynthesis. Abnormal increases in these processes presumably lead to uncontrolled cell division and growth, which results in vascular tissue destruction. In contrast, high concentrations of these herbicides inhibit cell division and growth, usually in meristematic regions that accumulate photosynthate assimilates and herbicide from the phloem. Auxin-mimicking herbicides stimulate ethylene evolution which may in some cases produce the characteristic epinastic symptoms associated with exposure to these herbicides.

5 **6** **7**

Photosystem II Inhibitors

Phenylcarbamates, pyridazinones, triazines, triazinones, uracils (Group 5), amides, ureas (Group 7), benzothiadiazinones, nitriles, and phenylpyridazines (Group 6), are examples of herbicides that inhibit photosynthesis by binding to the Q_B-binding niche on the D1 protein of the photosystem II complex in chloroplast thylakoid membranes. Herbicide binding at this protein location blocks electron transport from Q_A to Q_B and stops CO_2 fixation and production of ATP and $NADPH_2$ which are all needed for plant growth. However, plant death occurs by other processes in most cases. Inability to reoxidize Q_A promotes the formation of triplet state chlorophyll which interacts with ground state oxygen to form singlet oxygen. Both triplet chlorophyll and singlet oxygen can abstract hydrogen from unsaturated lipids, producing a lipid radical and initiating a chain reaction of lipid peroxidation. Lipids and proteins are attacked and oxidized, resulting in loss of chlorophyll and carotenoids and in leaky membranes which allow cells and cell organelles to dry and disintegrate rapidly. Some compounds in this group may also inhibit carotenoid biosynthesis (fluometuron) or synthesis of anthocyanin, RNA, and proteins (propanil), as well as effects on the plasmalemma (propanil) (Devine et al. 1993).

8 **16**

Fatty Acid and Lipid Biosynthesis Inhibitors

Benzofuranes (WSSA Group 16), phosphorodithioates (Group 8), and thiocarbamates (Group 8) are examples of herbicides that are known inhibitors of several plant processes including: 1) biosynthesis of fatty acids and lipids which may account for reported reductions in cuticular wax deposition, 2) biosynthesis of proteins, isoprenoids (including gibberellins), and flavonoids (including anthocyanins), and 3) gibberellin synthesis inhibition which may result from the inhibition of kaurene synthesis. Photosynthesis also may be inhibited (Gronwald 1991). A currently viable hypothesis that may link all these effects involves the conjugation of acetyl coenzyme A and other sulfhydryl-containing biomolecules by thiocarbamate sulfoxides (Casida 1974; Fuerst 1987). The sulfoxide forms may be the active herbicides (Ashton and Crafts 1981).

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Enolpyruvyl Shikimate-3-Phosphate (EPSP) Synthase Inhibitors

Glycines (glyphosate) are herbicides that inhibit 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase (Amrhein 1980) which produces EPSP from shikimate-3-phosphate and phosphoenolpyruvate in the shikimic acid pathway. EPSP inhibition leads to depletion of the aromatic amino acids tryptophan, tyrosine, and phenylalanine, all needed for protein synthesis or for biosynthetic pathways leading to growth. The failure of exogenous addition of these amino acids to completely overcome glyphosate toxicity in higher plants (Duke and Hoagland 1978; Lee 1980) suggests that factors other than protein synthesis inhibition may be involved. Although plant death apparently results from events occurring in response to EPSP synthase inhibition, the actual sequence of phytotoxic processes is unclear.

10

Glutamine Synthetase Inhibitors

Phosphinic acids (glufosinate and bialophos) inhibit activity of glutamine synthetase (Lea 1984), the enzyme that converts glutamate and ammonia to glutamine. Accumulation of ammonia in the plant (Tachibana 1986) destroys cells and directly inhibits photosystem I and photosystem II reactions (Sauer 1987). Ammonia reduces the pH gradient across the membrane which can uncouple photophosphorylation.

11 **12** **13** **27**

Carotenoid Biosynthesis Inhibitors

Amides, anilidex, furanones, phenoxybutan-amides, pyridiazinones, and pyridines (Group 12) are examples of compunds that block carotenoid biosynthesis by inhibition of phytoene desaturase (Bartels and Watson 1978; Sandmann and Böger 1989). Carotenoids play an important role in dissipating the oxidative energy of singlet O_2 ($^1O_2$). In normal photosynthetic electron transport, a low level of photosystem II reaction center chlorophylls in the first excited singlet state transform into the excited triplet state (^{3}Chl). This energized ^{3}Chl can interact with ground state molecular oxygen (O_2)to form $^1O_2$. In healthy plants, the energy of $^1O_2$ is safely quenched by carotenoids and other protective molecules. Carotenoids are largely absent in fluridone-treated plants, allowing $^1O_2$ and ^{3}Chl to abstract a hydrogen from an unsaturated lipid (e.g. membrane fatty acid, chlorophyll) producing a lipid radical. The lipid radical interacts with O_2 yielding a peroxidized lipid and another lipid radical. Thus, a self-sustaining chain reaction of lipid peroxidation is initiated which functionally destroys chlorophyll and membrane lipids. Proteins also are destroyed by $^1O_2$. Destruction of integral membrane components leads to leaky membranes and rapid tissue desiccation.

Callistemones, isoxazoles, pyrazoles, and triketones (Group 27) are examples of herbicides that inhibit *p*-hydroxyphenyl pyruvate dioxygenase (HPPD), which converts *p*-hydroxymethyl pyruvate to homogentisate. This is a key step in plastoquinone biosynthesis and its inhibition gives rise to bleaching symptoms on new growth. These symptoms result from an indirect inhibition of carotenoid synthesis due to the involvement of plastoquinone as a cofactor of phytoene desaturase.

Recent evidence suggests that clomazone (Group 13) is metabolized to the 5-keto form of clomazone which is herbicidally active. The 5-keto form inhibits 1-deoxy-D-xyulose 5-phosphate synthase (DOXP), a key component to plastid isoprenoid synthesis. Clomazone does not inhibit geranylgeranyl pyrophosphate biosynthesis (Croteau 1992; Weimer 1992).

Amitrole (Group 11) inhibits accumulation of chlorophyll and carotenoids in the light (Ashtakala, 1989), although the specific site of action has not been determined. Precursors of carotenoid synthesis, including phytoene, phytofluene, carotenes, and lycopene accumulate in amitrole-treated plants (Barry and Pallett 1990), suggesting that phytoene desaturase, lycopene cyclase, imidazoleglycerol phosphate dehydratase, nitrate reductase, or catalase may be inhibited. Other research (Heim and Larrinua 1989), however, indicates that the histidine, carotenoid, and chlorophyll biosynthetic pathways probably are not the primary sites of amitrole action. Instead, amitrole may have a greater effect on cell division and elongation than on pigment biosynthesis.

Aclonifen (Group 11) appears to act similar to carotenoid inhibiting/bleaching herbicides; but the exact mechanism of action in unknown.

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Protoporphyrinogen Oxidase (PPG oxidase or Protox) Inhibitors

Diphenylethers, *N*-phenylphthalimides, oxadiazoles, oxazolidinediones, phenylpyrazoles, pyrimidindiones, thiadiazoles, and triazolinones are herbicides that appear to inhibit protoporphyrinogen oxidase (PPG oxidase or Protox), an enzyme of chlorophyll and heme biosynthesis catalyzing the oxidation of protoporphyrinogen IX (PPGIX) to protoporphyrin IX (PPIX). Protox inhibition leads to accumulation of PPIX, the first light-absorbing chlorophyll precursor. PPGIX accumulation apparently is transitory as it overflows its normal environment in the thylakoid membrane and oxidizes to PPIX. PPIX formed outside its native environment probably is separated from Mg chelatase and other pathway enzymes that normally prevent accumulation of PPIX. Light absorption by PPIX apparently produces triplet state PPIX which interacts with ground state oxygen to form singlet oxygen. Both triplet PPIX and singlet oxygen can abstract hydrogen from unsaturated lipids, producing a lipid radical and initiating a chain reaction of lipid peroxidation. Lipids and proteins are attacked and oxidized, resulting in loss of chlorophyll and carotenoids and in leaky membranes which allows cells and cell organelles to dry and disintegrate rapidly (Duke 1991).

17 25 26

Potential Nucleic Acid Inhibitors or Non-descript mode of action

Several herbicides have been identified as having an unknown mode of action including organic arsenicals (Group 17), arylaminopropionic acids (Group 25), and other non-classified herbicides (Group 26).

18

Dihydropteroate Synthetase Inhibitors

The carbamate herbicide, asulam, appears to inhibit cell division and expansion in plant meristems, perhaps by interfering with microtubule assembly or function (Fedtke 1982; Sterrett and Fretz 1975). Asulam also inhibits 7,8-dihydropteroate synthase, an enzyme involved in folic acid synthesis which is needed for purine nucleotide biosynthesis (Kidd et al. 1982; Veerasekaran et al. 1981).

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19 **Auxin Transport Inhibitors**

Phthalamates (naptalam) and semicarbazones (diflufenzopyr) are compounds that inhibit auxin transport. These compounds inhibit polar transport of naturally occurring auxin, indoleacetic acid (IAA) and synthetic auxin-mimicking herbicides in sensitive plants. Inhibition of auxin transport causes an abnormal accumulation of IAA and synthetic auxin agonists in meristematic shoot and root regions, disrupting the delicate auxin balance needed for plant growth. When diflufenzopyr is applied with dicamba, it focuses dicamba's translocation to the meristematic sinks, where it delivers effective weed control at reduced dicamba rates and across a wider range of weed species. Sensitive broadleaf weeds exhibit rapid and severe plant hormonal effects (e.g., epinasty) after application of the mixture; symptoms are visible within hours, and plant death usually occurs within a few days. Symptomology, in sensitive annual grasses, is characterized by a stunted growth. Tolerance in corn occurs through rapid metabolism of diflufenzopyr and dicamba.

20 **21** **28** **29** **Cellulose Inhibitors**

Benzamides (WSSA Group 21), nitriles (Group 20), and triazolocarboxamides (Group 28) are herbicides that inhibit cell wall biosynthesis (cellulose) in susceptible weeds (Heim et al. 1990). Alkylazine (Group 29) herbicides inhibit cellulose biosynthesis (Myers et al. 2009).

22 **Photosystem I Inhibitors**

Bipyridyliums are examples of herbicides that accept electrons from photosystem I and are reduced to form an herbicide radical. This radical then reduces molecular oxygen to form superoxide radicals. Superoxide radicals then react with themselves in the presence of superoxide dismutase to form hydrogen peroxides. Hydrogen peroxides and superoxides react to generate hydroxyl radicals. Superoxides and, to a lesser extent, hydrogen peroxides may oxidize SH (sulfhydryl) groups on various organic compounds within the cell. Hydroxyl radical, however, is extremely reactive and readily destroys unsaturated lipids, including membrane fatty acids and chlorophyll. Hydroxyl radicals produce lipid radicals which react with oxygen to form lipid hydroperoxides plus another lipid radical to initiate a self-perpetuating chain reaction of lipid oxidation. Such lipid hydroperoxides destroy the integrity of cell membranes allowing cytoplasm to leak into intercellular spaces which leads to rapid leaf wilting and desiccation. These compounds can be reduced/oxidized repeatedly (Dodge 1982).

24 **Oxidative Phosphorylation Uncouplers**

Dinitrophenols (dinoterb) are herbicides that uncouple the process of oxidative phosphorylation causing almost immediate membrane disruption and necrosis.

NC **Not Classified**

These herbicides have not been classified by HRAC or WSSA.

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