UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MEMORANDUM

SUBJECT: Request for Phototoxicity Study Protocol for Light-Dependent Peroxidizing Herbicides

DATE: March 7, 2001

TO: Elizabeth Leovey, Acting Director
Environmental Fate and Effects Division
Office of Pesticide Programs

FROM: Norman B. Birchfield, Ph.D.
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The light-dependent peroxidizing herbicides (LDPHs) are a growing class of weed control chemicals (see partial listing attached). They act in plants by inhibiting the enzyme protoporphyrinogen oxidase (protox), which is the last common enzyme in the heme and chlorophyll biosynthetic pathways.[1] Protox exists in both plants and animals and the enzyme from both sources has been shown to be highly sensitive to many LDPHs.[2]

LDPH protox inhibition in plants results in a rapid accumulation of protoporphyrin IX, a phototoxic heme and chlorophyll precursor. In the presence of light, protoporphyrin IX is a powerful generator of singlet oxygen which in plants causes lipid membrane peroxidation leading to a rapid loss of turgidity and foliar burns. LDPH exposure in mammals has been shown to result in excretion of porphyrins in urine (porphynuria) and feces, increased liver weight, elevated blood porphyrin levels, developmental abnormalities, and cancer. Humans with a hereditary protox disorder (variegate porphyria) which results in lowered protox activity exhibit many symptoms similar to LDPH exposure in addition to photosensitivity. However, photosensitivity is not a commonly reported symptom of LDPH exposure in animals.

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[1]Matringe, M., J.-M. Camadro, P. Labbe, and R. Scalla. 1989. Protoporphyrinogen oxidase as a molecular target for diphenyl ether herbicides. *Biochem. J.* **260**: 231-235.

[2]Birchfield, N.B., and J.E. Casida. 1997. Protoporphyrinogen oxidase of mouse and maize: Target site selectivity and thiol effects on peroxidizing herbicide action. *Pesticide Biochemistry and Physiology* **57**, 36-43.

An LDPH-induced occurrence of phototoxicity in rats[3] and increased cytotoxicity to human skin cells grown in culture in the presence of light and an LDPH[4] have been reported but many other LDPH toxicity studies make no mention of phototoxicity in animals. The scarcity of phototoxicity data in animals could result from physiological or biochemical distinctions from plants. For instance, animals exposed to LDPHs may not normally accumulate protoporphyrin IX in their epidermis. However, phototoxicity may not be reported in many LDPH toxicity tests because of relatively low light conditions in laboratories and/or protection afforded by the animals' fur or feathers. Animals without fur or feathers existing in sunny environments would be expected to be at highest risk for potential phototoxic effects.

The Aquatic Biology Tech Team (ABTT) recommends that phototoxicity studies be conducted on herbicides with this mode of action to determine if animals exposed to LDPHs and intense light (similar to sunlight) show increased toxicity relative to controls exposed to LDPHs and low intensity light. The results of these studies will help to determine if animals that are exposed to sunlight in LDPH use areas are at higher risk than guideline toxicity studies suggest.

The ABTT is requesting that a LDPH phototoxicity protocol be submitted for review and agreement by EFED and the registrant prior to study initiation. Protocols for standard toxicity tests have also been published.[5] In nature, fish and other aquatic organisms are expected to be exposed to LDPHs through run-off and spray drift. Aquatic organisms inhabiting small, shallow water bodies, exposed to high levels of solar radiation would be expected to be at greatest risk for potential phototoxic effects. Therefore, the ABTT is requesting a small fish species be used in a phototoxicity assay to assess the potential of light to increase LDPH toxicity.

The ABTT requests that the study adequately address the following issues and suggests the paper, "Photoenhanced Toxicity of a Carbamate Insecticide to Early Life Stage Anuran Amphibians",[5] and other studies in the peer-reviewed scientific literature serve as sources of additional guidance:

Species
The fathead minnow may be an appropriate test species because of existing toxicity protocols which may be adapted for this study.

Exposure duration
A subchronic exposure duration would be adequate for proof of principle. A single exposure may

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[3]Halling, B.P., D.A. Yuhas, V.F. Fingar, and J.W. Winkleman. 1994. "Protoporphyrinogen oxidase inhibitors for tumor therapy" in *Porphyric Pesticides: Chemistry, Toxicology, and Pharmaceutical Applications*, (S.O. Duke and C.A. Rebeiz, Eds.) pp. 280-290, American Chemical Society Symposium Series 559, Am. Chem. Soc., Washington, D.C., 1994.

[4]Birchfield, N.B. *Protoporphyrinogen Oxidase as a Herbicide Target: Characterization of the [^{3}H]Desmethylflumipropyn Binding Site*. Dissertation. University of California, Berkeley. 1996.

[5]American Society for Testing and Materials. 1994. Standard guide for conducting the frog embryo teratogenesis assay-*Xenopus*. E 1439-91. In *Annual Book of ASTM Standards*, Vol 11.5, pp. 825-835. Philadelphia, PA.

not allow adequate time for porphyrin accumulation, however, a life-cycle is not necessary to identify a phototoxic effect.

Dosing
A range finding study should be conducted under defined low light conditions to identify an LC_{50} value and lower dose levels expected to be similar to controls. Doses used in the phototoxicity study should not be expected to result in significant mortality in low light controls. Dissolved concentrations of the test chemical should be confirmed by an appropriate analytical method.

Endpoints
Behavioral observations should be made in addition to measurements of mortality, growth, weight, morphology, and appearance. Ideally, measurements of protoporphyrin and heme concentrations in the blood and protox activity in the liver of each test organisms should be made.

Light sources
Artificial light may be preferred to natural light that will vary in different regions and seasons as well as with weather. If artificial light is used, the light should resemble full, natural sunlight as closely as possible, particularly around 400 nm. The most important wavelength for porphyrin induced phototoxicity in ~400 nm. No matter what the light source, the duration and intensity of UV and visible light should be reported at all wavelengths (200-800 nm). At this point EFED does not have a specific recommendation for an artificial light source.

Dark, light, and positive controls
As this study is intended to identify potential effects of light on LDPH toxicity, an appropriate study protocol should include a dark, or low light, control group. Another group not exposed to chemicals but exposed to full light should be included (a full light control). In addition to the dark and light controls, a positive control group using protoporphyrin IX may be useful.

Exposure chambers and light filters
Light intensity should be measured inside test chambers if glass or any other material is placed between the light source and the test animals. Any filters should be cured under the study light for 72-hours prior to study initiation to ensure consistent transmittance.

ATTACHMENT 1.

The following list of herbicides are believed to act by inhibiting protoporphyringen oxidase in the heme and chlorophyll biosynthetic pathway.

acifluorfen
azafenidin
carfentrazone-ethyl
flumiclorac-pentyl
flumioxazin
fluthiacet-methyl
fomesafen
lactofen
oxadiargyl
oxadiazon
oxyfluorfen
sulfentrazone
thidiazimin