



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON D.C., 20460

OFFICE OF  
CHEMICAL SAFETY AND  
POLLUTION PREVENTION

**MEMORANDUM**

**DATE: June 10, 2016**

**SUBJECT:** Guidance on Light-Dependent Peroxidizing Herbicides: Use of the Molar Threshold Value for Adjusting Fish Chronic Endpoints to Account for Ultraviolet Light-enhanced Toxicity

**FROM:** /s/ Anita Pease, Acting Division Director  
Environmental Fate and Effects Division (7507P)  
Office of Pesticide Programs

**TO:** Environmental Fate and Effects Division

The Aquatic Biology Technology Team (ABTT) has developed the following guidance for light-dependent peroxidizing herbicides (LDPHs) and other chemicals that may act via a light dependent peroxidizing (LDP) mode of action. The purpose of this guidance, which supersedes both the August 2010 interim guidance on light dependent peroxidizing herbicides<sup>1</sup> and the March 2016 guidance on light-dependent peroxidizing herbicides<sup>2</sup>, is to assist Environmental Fate and Effects Division (EFED) risk assessors in: (1) identifying herbicides and other pesticides that may act through a LDP mode of action; and (2) applying a molar threshold to fish chronic toxicity test endpoints in the absence of chemical-specific data to account for ultraviolet ( $uV$ ) light-enhanced toxicity.

## **1 Background**

The LDPHs are a class of herbicides that act in plants by inhibiting the enzyme protoporphyrinogen oxidase (protox), which is the last common enzyme in the chlorophyll biosynthetic pathways as well as in heme biosynthesis (Matringe, 1989)<sup>3</sup>. Protox inhibition in plants by LDPHs results in a rapid accumulation of protoporphyrin IX, a phototoxic precursor to chlorophyll and heme. In the presence of  $uV$  light, protoporphyrin IX can become a source of singlet oxygen, which in plants causes lipid membrane peroxidation leading to a rapid loss of

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<sup>1</sup> Brady, 2010. Interim Guidance on Light Dependent Peroxidizing Herbicides: Identification, DCI justification, and Use of a Toxicity Adjustment Factor on Fish Chronic Endpoints. Memorandum from Don Brady to EFED staff. August 20, 2010.

<sup>2</sup> Brady, 2016. Guidance on Light Dependent Peroxidizing Herbicides: Use of the Molar Threshold Value for Adjusting Fish Chronic Endpoints to Account for Ultraviolet Light-enhanced Toxicity. Memorandum from Don Brady to EFED staff. March 31, 2016.

<sup>3</sup> 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.

turgidity and foliar burns (Anderson *et al*, 1994)<sup>4</sup>. Protox exists in both plants and animals and has been shown to be highly sensitive to many LDPHs (Birchfield, 1997)<sup>5</sup>.

Several studies have documented enhanced toxicity of LDPHs to fish in the presence of *uV* light compared to toxicity observed under standard laboratory lighting (*e.g.*, MRIDs 42921601 and 48759101; 45389205 and 48409701; 44424201 and 46037001). Given that aquatic organisms are likely to be exposed simultaneously to LDPH and *uV* light in natural settings, concerns have been raised that standard laboratory tests may underestimate the toxicity of LDPH in shallow, clear waters. To address these concerns, the ABTT worked with a LDPH task force, consisting of multiple registrants, to establish a protocol for an early-life stage toxicity study (ELS; modified OCSPP Guideline 850.1400<sup>6</sup>) using enhanced *uV* lighting conditions. Three surrogate LDPH chemicals were tested, and the results of these studies were used to establish a molar threshold approach with which to adjust fish chronic toxicity endpoints to account for potential enhanced toxicity under enhanced *uV* lighting conditions. The molar threshold should be applied to the results of a standard ELS and full life cycle (LC; OCSPP Guideline 850.1500<sup>7</sup>) toxicity study for both freshwater and estuarine/marine fish for all LDPH chemicals if actual toxicity data under enhanced *uV* lighting conditions do not exist.

Guidance pertaining to identifying LDPHs and applying the molar threshold is provided below.

## 2 Identification of LDPH

A list of known LDPH chemicals as of 2015 is provided in **Attachment 1**. Most, but not all, LDPH chemicals have similar chemical structures, and these structures are presented in **Attachment 2**. It is possible that other currently registered chemicals act through the LDP mode of action. New active ingredients (*e.g.*, herbicides not previously registered by EPA) that act through the LDP mode of action may also be submitted for registration; therefore, it would be advantageous to identify these chemicals as possible protox-inhibitors during the pre-registration period. In order to recommend additional data and for the molar threshold to apply to these new chemicals, pertinent information about the mode of action for these chemicals must be obtained. It must be documented, either by registrant-submitted data or through open literature that the chemical acts by inhibiting protox. In addition to specific information on the LDP mode of action, other lines of evidence can be useful to identify protox-inhibiting chemicals such as certain hematological effects in mammals, *e.g.*, anemia, blood in stools, porphyria (an

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<sup>4</sup> Anderson, R. J., A. E. Norris, and F. D. Hess. Synthetic Organic Chemicals That Act through the Porphyrin Pathway. Porhyric Pesticides. April 15, 1994. 18-33. DOI:10.1021/bk-1994-0559.ch002

<sup>5</sup> 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.

<sup>6</sup> USEPA. 1996. Ecological Effects Test Guidelines. OCSPP 850.1400. Fish Early-Life Stage Toxicity Test. Office of Chemical Safety and Pollution Prevention (OCSPP) formerly the Office of Prevention, Pesticides and Toxic Substances (OPPTS) (7101). EPA 712-C-96-121. April 1996. <http://www.epa.gov/sites/production/files/2015-07/documents/850-1400.pdf>

<sup>7</sup> USEPA. 1996. Ecological Effects Test Guidelines. OCSPP 850.1500. Fish Life Cycle Toxicity. Office of Chemical Safety and Pollution Prevention (OCSPP) formerly the Office of Prevention, Pesticides and Toxic Substances (OPPTS) (7101). EPA 712-C-96-122. April 1996. <http://www.epa.gov/sites/production/files/2015-07/documents/850-1500.pdf>

accumulation of porphyrins in the blood). If an LDP mode of action is indicated, it is recommended that the ABTT be made aware of this finding in an effort to maintain a complete list of protox-inhibiting chemicals. Also, EFED risk assessors should ensure that the corresponding Health Effects Division (HED) toxicologist is made aware of this finding.

### 3 Origin and Use of the Molar Threshold

In 2006, EPA issued Data Call-Ins (DCIs) requiring fish ELS toxicity tests to be conducted under high intensity  $uV$  lighting for known LDPHs. In response to the DCI, LDPH registrants formed a task force and agreed to test three LDPH chemicals to generate empirical data that could serve as a surrogate for untested LDPHs. The three surrogate LDPHs (*i.e.*, oxadiazon, oxyfluorfen, and pyraflufen-ethyl) were selected for testing on the basis of toxicity and chemical-physical characteristics (*i.e.*, octanol-water partition coefficient [ $K_{ow}$ ] and photolysis half-life).

In 2010, an interim 29-fold adjustment factor was implemented<sup>1</sup> for the LDPH chemical class lacking experimentally-derived toxicity data under high intensity  $uV$  lighting conditions. The interim factor of 29x was developed by the ABTT using the most conservative data available (*i.e.*, ELS data for oxyfluorfen calculated as the ratio of standard light toxicity to high intensity  $uV$  light toxicity).

Based on a review of the data from the three surrogate chemicals, the adjustment factor method used for the interim measure (29X) does not sufficiently capture the wide variability of the toxicity effects of the untested LDPH chemicals. For the three surrogate chemicals, there was a high degree of variability between the ratio of the no observed adverse effect concentrations (NOAECs) from the standard light and high intensity  $uV$  light studies (*i.e.*, the ratio ranged from approximately 1 to 400). This observed variability within a limited dataset resulted in uncertainties in using the interim, single-value adjustment factor based on the ratio of standard-lighting to  $uV$  exposure toxicity data alone.

After reviewing the available LDPH fish chronic toxicity data, the EFED Science Policy Panel (SPP) concluded that:

- 1) high intensity  $uV$  exposure elicits a greater toxic response than standard light exposure for LDPH chemicals;
- 2) the relationship between LDPH toxicity under standard light and LDPH toxicity under high intensity  $uV$  varies considerably among the three surrogate chemicals (ratios ranging from 1-400);
- 3) because of this variability, the standard light and high intensity  $uV$  study endpoints are not related in a statistically robust manner; and
- 4) it is therefore not appropriate to use a single adjustment factor applied to standard light exposure endpoints to predict the  $uV$ -enhanced toxicity of a LDPH.

The SPP recommended the use of molar equivalency to derive NOAEC values for fish based on a molar threshold approach (described in **Section 3.1**). In the absence of chemical-specific data on LDPH toxicity under high intensity  $uV$  lighting, EFED risk assessors should apply a molar threshold of 0.002  $\mu\text{moles/L}$  to freshwater and estuarine/marine fish ELS and LC toxicity endpoints from studies conducted under standard laboratory lighting for risk estimation.

The molar threshold NOAEC accounts for the potential enhanced toxicity of LDPH chemicals under natural sunlight. The molar threshold approach is based on the observation that regardless of the NOAEC value determined under standard laboratory lighting for the three surrogate chemicals, the effect level under high intensity  $\mu V$  lighting conditions was relatively consistent (*i.e.*, 0.002 to 0.02  $\mu\text{moles/L}$ ). It is noted that the data supporting the molar threshold (0.002  $\mu\text{moles/L}$ ) are limited to a single species (*i.e.*, fathead minnows; *Pimephales promelas*) and three chemicals and may not reflect the extent of variability in  $\mu V$ -enhanced toxicity across species and chemicals. The molar threshold, however, is conservatively applied to any ELS or LC endpoints (*e.g.*, hatch, larval survival, post-hatch survival), while the threshold itself is derived using a dry weight NOAEC. The use of a molar threshold is not new; other EFED risk assessments have relied on a molar threshold to evaluate a class of chemicals such as the dioxin toxicity evaluation presented in the 2,4-D Reregistration Eligibility Decision<sup>8</sup>.

### 3.1 Steps in Developing the Molar Threshold Approach

#### 3.1.1 Step 1: Establish a $\mu V$ lower-limit molar correction

The molar correction was calculated by taking the lowest NOAEC value (for all available endpoints for the three surrogate chemicals) divided by the molecular weight (MW) of the chemical (**Table 1** and **Equation 1**). The molar threshold is the lowest of the molar-corrected NOAECs, 0.002  $\mu\text{moles/L}$ , the MW-corrected dry weight NOAEC for pyraflufen-ethyl.

#### Equation 1.

$$\frac{\text{Endpoint NOAEC}}{\text{Molecular weight}} = \text{Molecular weight correction}$$

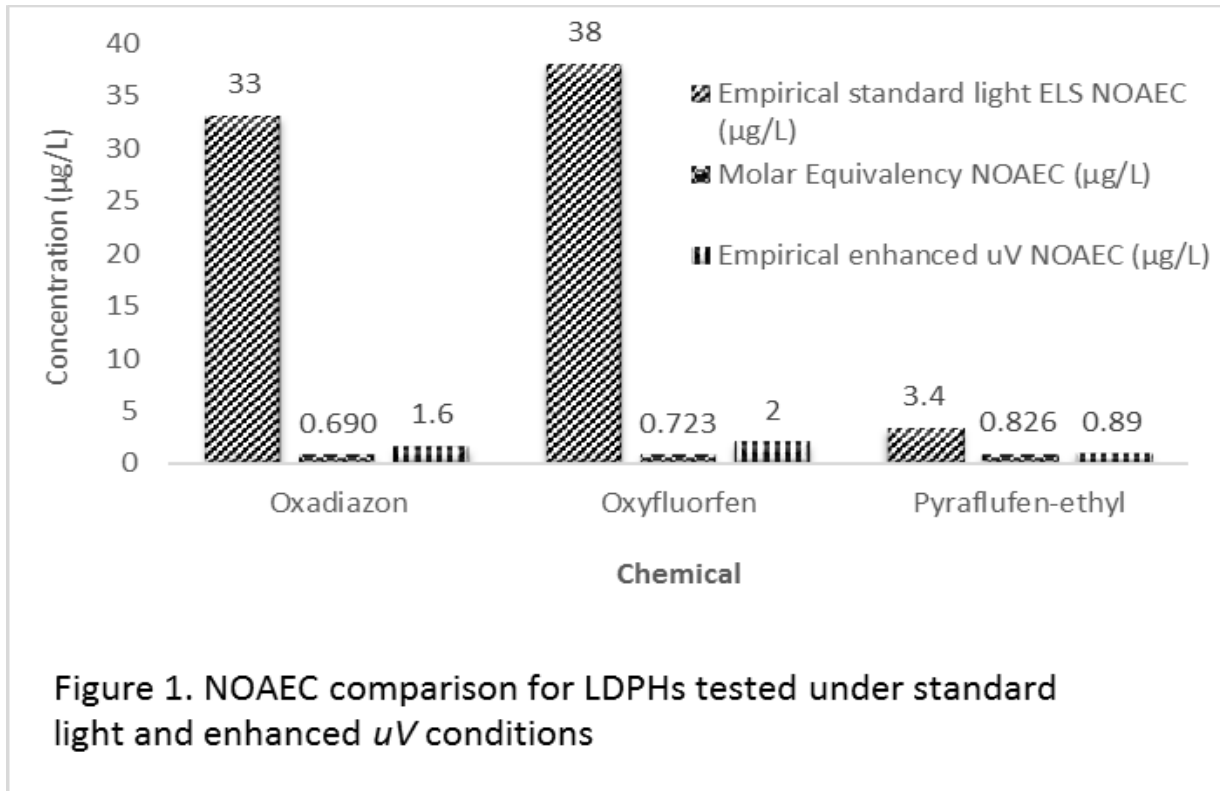
**Table 1.** Endpoint specific NOAECs and Molecular Weight (MW) adjusted NOAECs for the three LDPHs tested under enhanced  $\mu V$  lighting

	MW (grams/mole)	Hatch NOAEC ( $\mu\text{g/L}$ )	MW adjusted NOAEC ( $\mu\text{mol/L}$ )	Larval Survival NOAEC ( $\mu\text{g/L}$ )	MW adjusted NOAEC ( $\mu\text{mol/L}$ )	Post-Hatch Survival NOAEC ( $\mu\text{g/L}$ )	MW adjusted NOAEC ( $\mu\text{mol/L}$ )	Dry Weight NOAEC ( $\mu\text{g/L}$ )	MW adjusted NOAEC ( $\mu\text{mol/L}$ )	Length NOAEC ( $\mu\text{g/L}$ )	MW adjusted NOAEC ( $\mu\text{mol/L}$ )
<b>Oxadiazon</b>	345.2										
Enhanced $\mu V$ NOAEC		1.6	0.005	1.6	0.005	7.6	0.022	3.8	0.011	3.8	0.011
<b>Oxyfluorfen</b>	361.7										
Enhanced $\mu V$ NOAEC		2.2	0.006	4.2	0.012	1.7	0.005	3.3	0.009	3.3	0.009
<b>Pyraflufen-ethyl</b>	413.2										
Enhanced $\mu V$ NOAEC		6.9	0.017	3.5	0.008	3.5	0.008	0.89	0.002*	1.6	0.004

\* The molar threshold is the lowest of the molar-corrected NOAECs, 0.002  $\mu\text{moles/L}$ , the MW-corrected dry weight NOAEC for pyraflufen-ethyl

<sup>8</sup> Reregistration Eligibility Decision for 2,4-D. Office of Prevention, Pesticides, and Toxic Substances. June 2005. Pages 82-83 of 304. Available online at: [http://archive.epa.gov/pesticides/reregistration/web/pdf/24d\\_red.pdf](http://archive.epa.gov/pesticides/reregistration/web/pdf/24d_red.pdf)

The three LDPHs selected for testing under enhanced  $uV$  light (which were also previously conducted under standard laboratory light) indicate that a molar correction applied to the standard light derived NOAEC will yield a conservative estimate of toxicity under enhanced  $uV$  conditions (**Figure 1**). Oxadiazon, oxyfluorfen, and pyraflufen-ethyl tested under standard laboratory lighting each share dry weight as the most sensitive endpoint, facilitating the comparison depicted in **Figure 1**.



### 3.1.2 Step 2: Calculate Chemical-Specific NOAEC for Untested Chemical

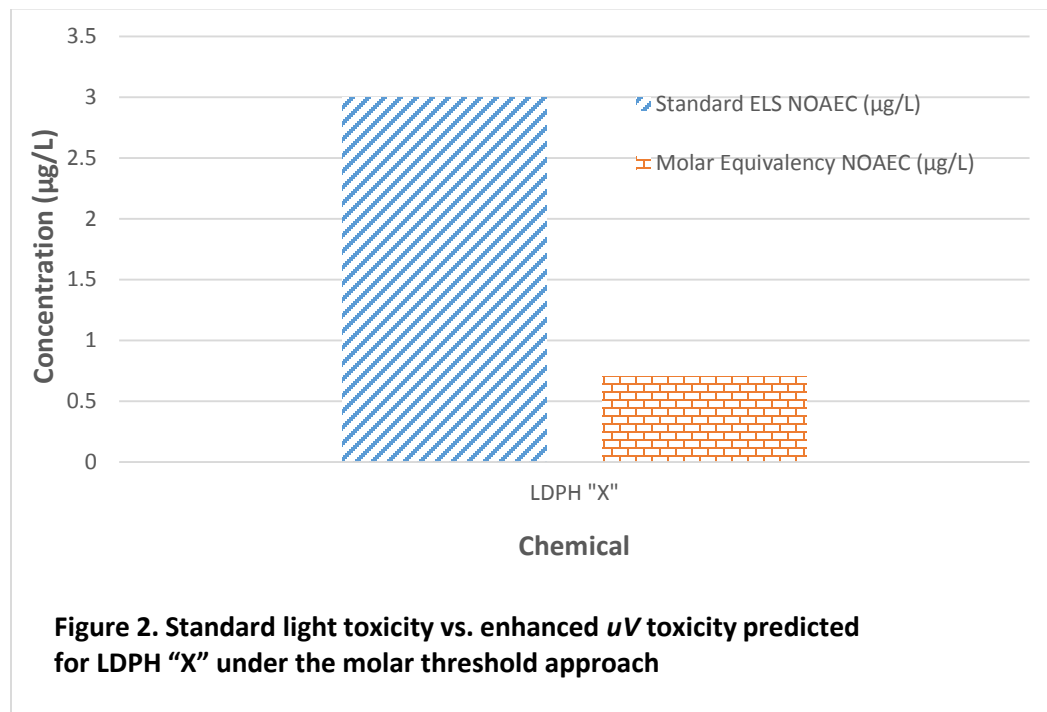
To obtain a chemical-specific NOAEC, the surrogate molar threshold is adjusted based on the MW of the desired chemical. **Equation 1** can be rearranged to calculate the toxicity of a LDPH not tested under high intensity  $uV$  conditions using the MW of the untested chemical and the molar threshold (the lowest MW-corrected NOAEC of the available data) (**Equation 2**).

#### **Equation 2.**

$$\text{Molar Equivalency NOAEC} = \text{Molar threshold} * \text{Molecular weight}$$

For example, consider a scenario with LDPH “X” where a standard light fathead minnow ELS test determined a NOAEC of 3 µg/L based on statistically significant effects to length and weight. LDPH “X” has a MW of 350 grams/mole, and the surrogate molar threshold is 0.002

$\mu\text{mol/L}$ . Rearranging the molar threshold equation and multiplication of the LDPH “X” MW (350 grams/mole) by the molar threshold ( $0.002 \mu\text{mol/L}$ ) yields a MW-corrected NOAEC of  $0.7 \mu\text{g/L}$ <sup>9</sup>, which serves as the chronic fish toxicity NOAEC value under enhanced  $uV$  exposure (Figure 2).



### 3.2 NOAEC Values for Use in Risk Assessment

Since the MWs of all LDPH chemicals are relatively consistent (range *ca.* 30%; see **Attachment 1** for listing of known LDPHs and associated MWs) across the class, the chemical-specific molar threshold-based NOAECs are relatively similar for all LDPHs (**Table 2**). Empirically-derived NOAEC values for the three tested surrogate chemicals are presented in **Table 3**.

<sup>9</sup>  $LDPH \text{ “X” Molar Equivalency NOAEC} = 0.002 \mu\text{mol/L} * 350 \text{ g/mol}$   
 $LDPH \text{ “X” Molar Equivalency NOAEC} = 0.7 \mu\text{g/L}$

**Table 2.** LDPH molar threshold NOAECs

Chemical	Enhanced <i>uV</i> Molar Threshold NOAEC (µg/L)
Carfentrazone-ethyl	0.82
Flufenpyr-ethyl	0.82
Flumiclorac-pentyl	0.85
Flumioxazin	0.71
Fluthiacet-methyl	0.81
Fomesafen	0.88
Lactofen	0.92
Sodium acifluorfen	0.72
Sulfentrazone	0.77

**Table 3.** Empirically-derived NOAECs under *uV* Conditions for the Three Surrogate Chemicals

Chemical	Enhanced <i>uV</i> Empirically-derived NOAEC (µg/L)
Oxadiazon	1.6
Oxyfluorfen	2.0
Pyraflufen-ethyl	0.89

#### **4 Use of the NOAEC (by either molar threshold or empirically-based under enhanced *uV* conditions) in Ecological Risk Assessment**

##### **4.1 How should the Risk Quotient (RQ) value be calculated for a specific chemical?**

Two RQs should be calculated:

- 1) To represent the phototoxic potential of the LDPH class  

$$\text{RQ} = \text{EEC (60-day average 1-in-10 year)} \div \text{NOAEC (molar equivalency or enhanced } uV \text{ conditions)}$$
- 2) To represent lower *uV* light scenarios,  

$$\text{RQ} = \text{EEC (60-day average 1-in-10 year)} \div \text{NOAEC (determined under standard laboratory lighting)}$$

The calculation of two RQs is meant to provide an evaluation of potential risk under a range of *uV* conditions (*i.e.*, bounding high and low *uV* exposure). The first RQ is meant to be representative of environmental conditions under higher *uV* light exposure such as clear, shallow water bodies, whereas the second RQ is meant to be representative of conditions where *uV* light exposure potential is low such as turbid or deeper waters.

## 4.2 Which Level of Concern (LOC) should be the basis for RQ comparison?

Both RQs should be compared to the chronic risk, non-listed and listed level of concern (LOC) of 1.0.

## 4.3 Are toxicity data for other taxa adjusted using the molar threshold?

At this time, with the information available, the ABTT and SPP are recommending that the molar threshold should only be applied to freshwater and estuarine/marine fish ELS and LC NOAEC values. If further information becomes available indicating the need for an adjustment factor for other taxa, it will be addressed at that time. Consistent with EFED's risk assessment paradigm, freshwater fish are surrogates for aquatic-phase amphibians.

## 5 Data Needs for Ecological Risk Assessment<sup>10</sup>

For LDPHs without a fish ELS study conducted under standard lighting, that study should still be requested for two reasons:

- 1) the standard light endpoint can be used for species that are not likely to encounter  $\mu V$  radiation (*e.g.*, turbid, deep waters; endangered species assessments for cave-dwelling fish species) and;
- 2) to ensure that the NOAEC under standard laboratory lighting is not less than the lowest known standard light NOAEC for the class such that the molar threshold would no longer be a conservative estimate of  $\mu V$ -enhanced toxicity.

If the molecular weight corrected standard light ELS or LC NOAEC is greater than the  $\mu V$  molar threshold (0.002  $\mu\text{mol/L}$ ), proceed with the molar threshold approach. However, if the MW-corrected standard light ELS or LC NOAEC falls at or below the  $\mu V$  lower-limit threshold of 0.002  $\mu\text{mol/L}$ , a fathead minnow ELS study under  $\mu V$  exposure should be requested<sup>11</sup>. This additional testing recommendation is predicated on the assumption that testing from the three surrogate chemicals under  $\mu V$  exposure has identified the lowest molar threshold for the LDPH class.

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<sup>10</sup> Fish life cycle (LC) studies are conditionally required based on multiple factors specified in 40 CFR §158.630. It is not anticipated that the Office of Pesticide Programs will be requesting fish LC studies conducted under enhanced  $\mu V$  conditions at this time.

<sup>11</sup> Endpoints derived from fathead minnow ELS testing under enhanced  $\mu V$  conditions will be treated as a surrogate for estuarine/marine fish ELS endpoints under an enhanced  $\mu V$  exposure scenario in the absence of additional information.



## 6 Potential Hazard Label Language

If the risk assessment identifies potential adverse effects to fish exposed to a LDPH under  $uV$  conditions, the following hazard label language might be considered. It is recommended that EFED discuss any potential hazard label language with the Registration Division(s), particularly if any modifications to the proposed language below may be needed to address a LDPH chemical-specific situation.

*This product may be hazardous to aquatic organisms, particularly in clear, shallow water bodies that are adjacent to treated areas. Therefore, transport to water by runoff or spray drift of this product in areas where surface water is present, or intertidal areas below the mean high water mark should be avoided. Do not contaminate water when disposing of equipment washwater or rinsate.*

## 7 Changes from the 2010 Interim Adjustment Factor Memo

The 2010 interim guidance memo states:

*For the new registration of LDPHs, two early life stage fish studies (i.e. the 850.1400 and the modified light ELS study) will not be required. An early life stage fish study with modified lighting (i.e., enhanced  $uV$ ) will be sufficient to satisfy the guideline requirement provided that a low light "reference treatment" is used in which the highest treatment concentration is tested.*

At this time, EFED considers toxicity data from testing under  $uV$  exposure (either by using the molar threshold approach discussed in this memo or chemical-specific  $uV$  exposure testing) and a standard light study for each chemical in the LDPH class as necessary data.

## 8 Uncertainties

The ABTT acknowledges that there are uncertainties associated with the molar threshold value that may underestimate or overestimate the actual toxicity to fish under natural sunlight in the environment. These uncertainties include the following:

- Exposure to  $uV$  in the laboratory studies is relatively constant over time; whereas, temporal variability in  $uV$  exposure in the field is expected to be high.
- The magnitude of  $uV$ -enhanced toxicity for fish in the natural environment may differ substantially between the laboratory and the field and will likely depend on the interaction of  $uV$  exposure with the timing and location of reproduction and hatching events in addition to factors affecting  $uV$  light attenuation in the natural environment.
- The extent to which compensatory mechanisms offset the potential for phototoxic effects in the natural environment is uncertain.
- The data supporting the molar threshold are limited to a single species and three chemicals, which may not capture the extent of variability in  $uV$  enhancement across all non-target species and LDP chemicals.

Furthermore, the ABTT notes that enhanced sensitivity to LDPHs is not limited to fish and could be observed in other taxa such as aquatic invertebrates and terrestrial species (*e.g.*, birds, terrestrial-phase amphibians, reptiles and mammals)<sup>12</sup>. Indicators of toxicity from LDPHs in toxicity tests with other taxa could be observed by the appearance of blood in stool, porphyria, or other observations. Reviewers should be aware of potentially higher toxicity under increased *uV* and consider its effects when determining risk to organisms. However, at this time, with the information available, the ABTT is recommending that the molar threshold should be applied only to freshwater and estuarine/marine fish ELS and LC NOAEC values. If further information becomes available indicating the need for an adjustment factor for other taxa, it will be addressed at that time.

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<sup>12</sup> 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.

## Attachment 1

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

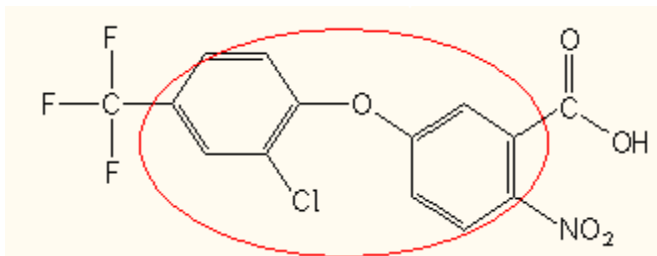
<b>Chemical name</b>	<b>Molecular weight (g/mol)*</b>
Azafenidin	338.2
Carfentrazone-ethyl	412.2
Flufenpyr-ethyl	408.7
Flumiclorac-pentyl	423.9
Flumioxazin	354.3
Fluthiacet-methyl	403.9
Fomesafen	438.8
Lactofen	461.8
Oxadiargyl	341.2
Oxadiazon	345.2
Oxyfluorfen	361.7
Pyraflufen-ethyl	413.2
Sodium acifluorfen	361.7
Sulfentrazone	387.2
Thidiazimin	372.4

\*Molecular weights as reported in the University of Hertfordshire Pesticides Properties Database.  
Available online at: <http://sitem.herts.ac.uk/aeru/ppdb/en/atoz.htm>

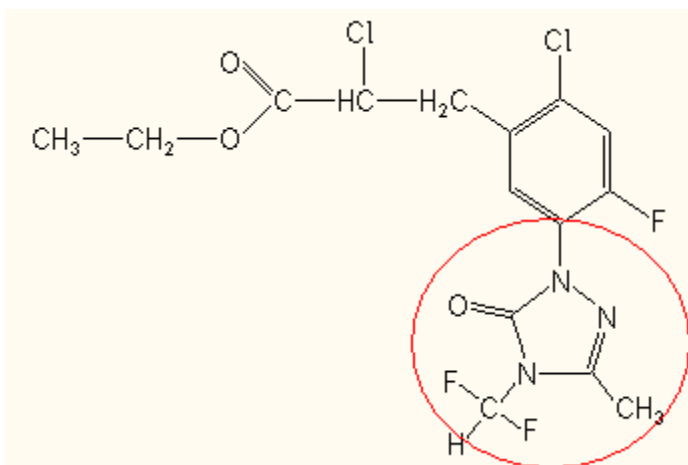
## Attachment 2

LDPH chemicals are found in a variety of chemical classes; therefore, the class of chemical cannot necessarily be used to help identify potential LDPH chemicals. However, the LDPH chemicals tend to share similarities in their chemical structure. The following characteristics are shared by most (but not all) LDPH chemical and therefore represent a good place to start.

- 1) Does it have a diphenyl ether (**Fig. 1a**) or an N-phenyl heterocycle (**Fig. 1b**)?

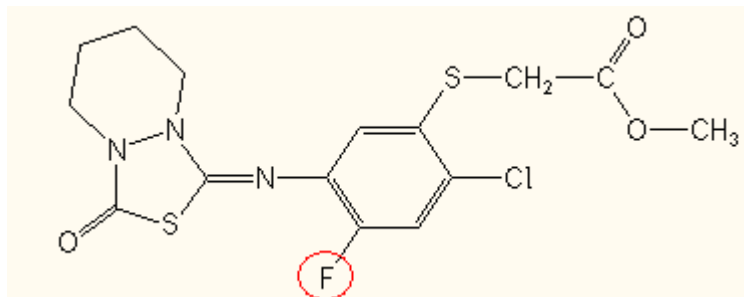


**FIGURE 1a.** Example of a diphenyl ether.

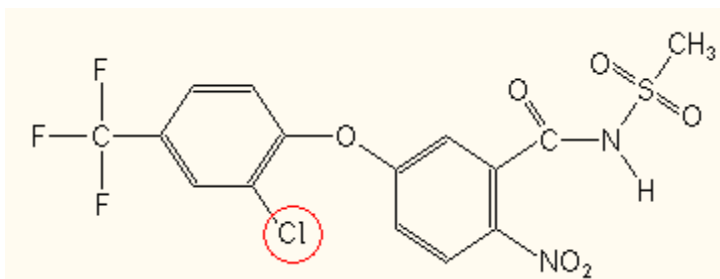


**FIGURE 1b.** Example of an N-phenyl heterocycle.

- 2) On the phenyl ring, is there a fluorine (**Fig. 2a**) or chlorine (**Fig. 2b**) in the *ortho*-position relative to the ether or heterocycle?

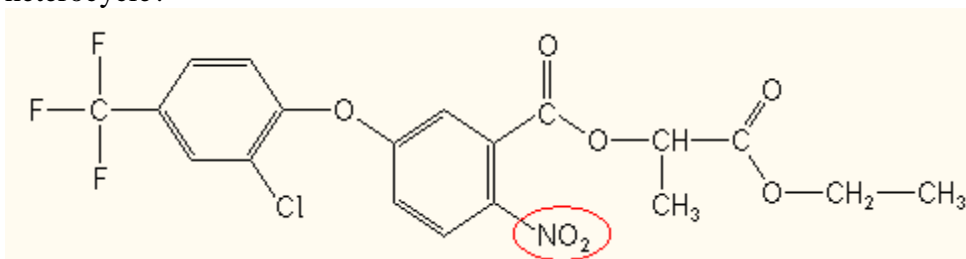


**FIGURE 2a.** Example of a fluorine in the *ortho*-position.

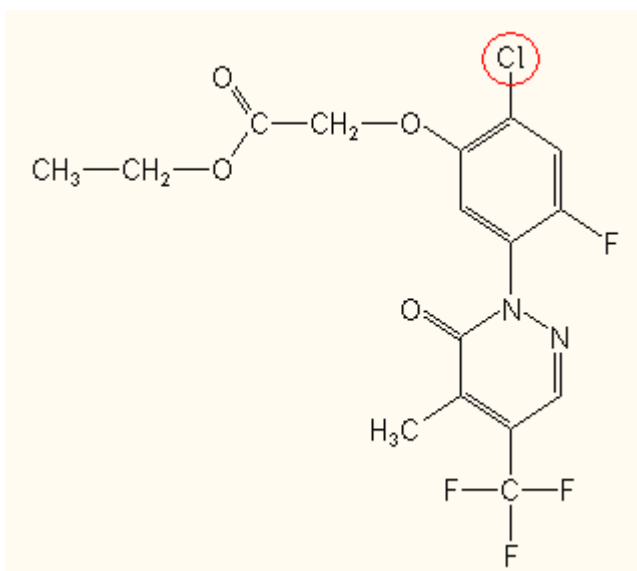


**FIGURE 2b. Example of a chlorine in the *ortho*-position.**

- 3) Is there a nitro (**Fig. 3a**) or chloro (**Fig. 3b**) in the *para*-position relative to the ether or heterocycle?

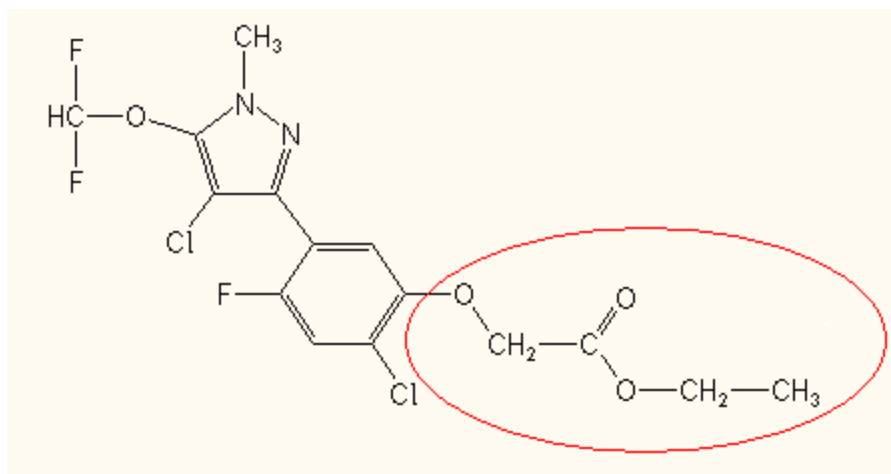


**FIGURE 3a. Example of a nitro in the *para*-position.**

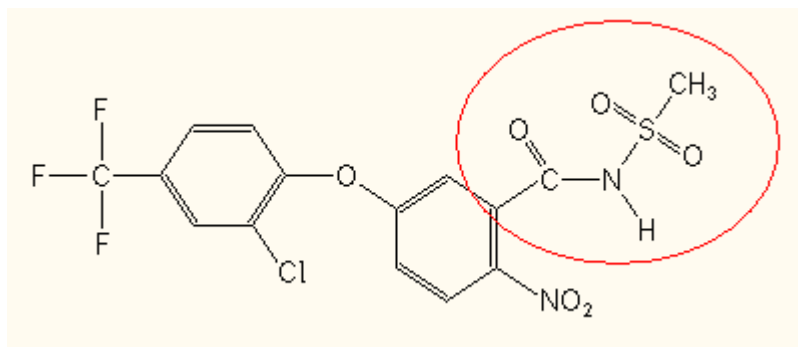


**FIGURE 3b. Example of a chloro in the *para*-position.**

- 4) Is there a complex chain in the *meta*-position from the heterocycle (**Fig. 4a**) or ether (**Fig. 4b**)?



**FIGURE 4a.** Example of a complex chain in the *meta*-position relative to the heterocycle.



**FIGURE 4b.** Example of a complex chain in the *meta*-position relative to the ether.