

Environmental Fate and Toxicology of Oxyfluorfen

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1 Introduction

Oxyfluorfen [2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluoromethyl) benzene] (Fig. 1) is nitrodiphenyl ether herbicide first marketed by Rohm and Haas Co. in 1976 (Shaner 2014) and registered by the U.S. Environmental Protection Agency (EPA) in 1979 (US EPA 2002). It is applied to control pre-emergent and post-emergent grassy and broadleaf weeds with major crop uses on wine grapes, almonds, and cotton; the major non-crop uses including nursery ornamentals and forestry in California, Washington, Oregon, and along the Mississippi River. In 2015, roughly 882,070 lbs active ingredient (a.i.) were applied in California (CDPR 2015).

Due to the persistence and toxicity of oxyfluorfen, concerns exist regarding its potential to contaminate the environment and impact non-target organisms. Oxyfluorfen has the potential to contaminate surface water through runoff and spray drift (Alister et al. 2009). It is highly toxic to aquatic plants and fish, and moderately to highly toxic to aquatic invertebrates (US EPA 2002). This paper provides a literature overview of the chemistry, environmental fate, and toxicology of oxyfluorfen to better understand and prevent potential environmental impacts.

2 Chemistry

Oxyfluorfen is a nitrodiphenyl ether herbicide with a structure similar to that of nitrofen and acifluorfen (Keum et al. 2008). Technical grade oxyfluorfen occurs as a red-brown to yellow semi-solid at room temperature with a faint smoky scent (Shaner 2014). It is readily soluble in most organic solvents but is poorly soluble in water (0.116 mg/L) and has a high tendency to bind to soil containing organic matter (US EPA 2002). It is stable up to 50°C. Degradation is

primarily via photolysis, whereas hydrolysis plays no significant role (US EPA 2001b). The physicochemical properties of oxyfluorfen are presented in Table 1.

3 Chemodynamics

3.1 Soil

Due to its high K_{ow} and K_{oc} values, oxyfluorfen strongly adsorbs to soil particles and is relatively immobile within the soil profile (Alister et al. 2009; Shaner 2014). Oxyfluorfen is particularly retained in soils with high organic matter or clay content (Shaner 2014) and generally considered persistent.

Several researchers have calculated soil-sorption coefficients and observed the mobility of oxyfluorfen within a variety of soil types. Alister et al. (2009) conducted a four-year study of oxyfluorfen (0.75 kg a.i./ha; bare sandy loam soil) adsorption and dissipation under both irrigated (30 mm H₂O for every 15 days during the first 90 days post-application) and natural rainfall conditions. Authors found more than 74% of the oxyfluorfen remained in the top 2.5 cm of the studied soil at 90 and 340 days post-application and was not detected below 10 cm (Alister et al. 2009). In a soil column experiment with Taiwanese soils (clay loam, silty clay, sandy clay loam, and loam), Yen et al. (2003) reported similar results: the a.i. remained primarily within a 0 to 3 cm soil depth. The authors further determined the soil/liquid partitioning coefficient (K_d) and percent organic carbon as 52 mL/g, 0.33% for loam, 151 mL/g, 1.05% for sandy clay loam, 421 mL/g, 1.62% for silty clay, 613 mL/g, 2.32% for clay loam, and 755 mL/g, 2.06% for silty clay. The results indicate that oxyfluorfen's affinity to soil generally increased with increased organic content.

The U.S. EPA (2002) reported oxyfluorfen to have aerobic soil half-lives of 291-294 days for clay loam soils and 556-596 days for sandy loam soils. However, other studies have reported shorter half-lives under different environmental conditions. In Taiwan, a field study of oxyfluorfen dissipation in soils of varied moisture contents and temperatures reported half-lives ranging from 72 to 60 days (Yen et al. 2003). The authors also studied the influence of soil temperature on the dissipation of oxyfluorfen, and found that its dissipation increased as temperature increased (Yen et al. 2003). A study conducted in the Barossa Valley of South Australia reported a soil half-life of 119 days for a sandy soil in a Mediterranean type climate (Ying & Williams 2000). Although the half-lives vary, each author indicates that oxyfluorfen is moderately to strongly persistent in soils.

3.2 Water

Oxyfluorfen is considered a risk for surface water contamination via runoff or erosion due to its strong affinity for organic matter (Alister et al. 2009; US EPA 2001b; Yen et al. 2003). Mantzos et al. (2014) investigated oxyfluorfen runoff from a silty clay soil cropped with sunflowers or left fallow. Over the 191- day trial, more oxyfluorfen was transported via sediment from fallow plots than from plots with sunflowers.

In recent years, the California Department of Pesticide Regulation's (CDPR) Surface Water Protection Program has frequently detected oxyfluorfen in agricultural waterways in Monterey, San Louis Obispo, Santa Barbara, and Imperial counties. In these waterways, oxyfluorfen was detected in 41% of the samples in 2016 (Deng 2017); three samples exceeded the lowest U.S.

EPA aquatic life benchmark value (nonvascular plant, acute = 0.29 µg/L; USA EPA 2017).

Detections of oxyfluorfen were also found in urban waterways in Alameda, Contra Costa, Placer, Sacramento, and Santa Clara counties. Oxyfluorfen was detected in 6% of the samples in 2016, and one sample exceeded the lowest aquatic life benchmark value (Ensminger 2016). Although it is rare to see oxyfluorfen concentrations that exceed the lowest aquatic life benchmark, its co-appearance in surface water with other contaminants could have additive or synergistic effects on non-target aquatic species (Deng 2017).

Oxyfluorfen poses a low-risk as a groundwater contaminant mainly due to its low solubility in water and strong affinity to soil; however, the potential of its leaching into groundwater exists (Alister et al. 2009; US EPA 2001b; Yen et al. 2003). Studies on oxyfluorfen leaching have identified the conditions under which groundwater contamination may occur. Janaki et al. (2013) conducted a soil column leaching study with sandy clay loam soil treated with two concentrations of technical grade oxyfluorfen. Total leachate concentrations ranged from 0.9 to 1.2% for the 0.2 kg a.i./ha and 0.4 kg a.i./ha applications, respectively. Yen et al. (2003) also predicted the potential for oxyfluorfen to contaminate aquifers in Taiwan by using the groundwater pollution-potential model. After consideration of the remaining residue and travel time (leaching rate, 0.6 cm/day), the model revealed that under normal conditions oxyfluorfen is not very mobile in soil and may not contaminate groundwater.

3.3 Air

Loss of oxyfluorfen through volatilization is limited as its low vapor pressure and Henry's law constant values suggest (Table 1; Shaner 2014). In Parlier, California, CDPR conducted an ambient air study to monitor oxyfluorfen and 39 other pesticides and degradation products.

During this study air samples were taken weekly for 3 consecutive days per week for a duration of one year using columns packed with XAD-4 resin at a flow rate of 15 L/min. CDPR collected 468 samples that were analyzed for oxyfluorfen; none of them above the method detection limit of 6.39 ng/m³ (Wofford et al. 2014).

Although oxyfluorfen volatility is low, drift during application may occur. In the Centre Region of France, Coscollà et al. (2010) analyzed ambient air samples collected from three rural and two urban sites. Of the 41 detected pesticides, oxyfluorfen was detected at a frequency of 3%, and its concentration ranged from 0.65 to 3.01 ng/m³. Since oxyfluorfen was detected at all sampling sites the authors concluded that it persists in the atmosphere long enough for local and regional transport despite the herbicide's low atmospheric half-life ($t_{1/2} = 12$ hr; Coscollà et al. 2010).

4 Environmental Degradation

4.1 Abiotic Processes

4.1.1 Hydrolysis

Oxyfluorfen is resistant to hydrolysis in the environment (Shaner 2014; US EPA 2001b) (Table 1). To illustrate, US EPA (2001b) reviewed a hydrolysis study in acidic and basic aqueous buffered pH 4, 7, 10 solutions using 50 µg a.i./L concentration conducted in the dark at 25°C and 45°C. After 30 days, the results concluded that oxyfluorfen is stable to hydrolysis at pH 4, 7, 10, and that hydrolysis is not a major degradation route. US EPA (2001b) reviewed one additional study with similar results when using a concentration of 50 µg a.i./L of oxyfluorfen aqueous buffered solutions at pH 4, 7, 10.

4.1.2 Photolysis

Photochemical reactions play a major role in the abiotic degradation of oxyfluorfen. Scrano et al. (1999) confirmed photolysis in various solvents (acetonitrile, methanol, and *n*-hexane; 1mM oxyfluorfen). The reaction was stopped after 20% of the a.i. was degraded with either a mercury arc lamp (125 W; 21°C) or a xenon lamp (1100 W; 21°C). Of the tested solvents, degradation occurred more rapidly in methanol under the mercury arc lamp and in acetonitrile under the xenon lamp. With further analysis of the acetonitrile solvent, Scrano et al. (1999) identified four degradation products after 24 hr of irradiation (Figure 2). Thomas et al. (2009) studied the photodegradation of oxyfluorfen on the surface of aluminum sheets and soil under simulated sunlight conditions. Using plasma desorption time-of-flight mass spectrometry The authors found oxyfluorfen degradation was faster when sunlight was unfiltered compared to UV-filtered sunlight.

In natural environments, photolysis primarily occurs in water and on soil surfaces. Ying and Williams (1999) compared the photo degradative half-life of oxyfluorfen in Milli-Q® water and sterile soil under sunlight for 12 days. The authors found half-lives to be much shorter in water than on soil (5 hr vs 5.19 d, respectively). Slower degradation in soil is due to light attenuation, which limits direct photolysis to a 1-mm soil depth (Ying & Williams 1999).

4.2 Biotic Processes

Microbial degradation is the major pathway for oxyfluorfen degradation in soils. Keum et al. (2008) investigated the degradation of oxyfluorfen (1000 mg/L; nutrient broth) using the bacteria *Sphingomonas wittichii*. After a 7-day exposure, 75% of the oxyfluorfen was degraded and two

main degradates (desethyl N-acetylaminooxyfluorfen and 3-ethoxy-4-nitrophenol) were produced through (1) reduction, and (2) N-acetylation of the nitro groups and ether bond cleavage (Keum et al. 2008). The bacteria *Azotobacter chroococcum*, exposed to a sterilized nutrient salt solution containing oxyfluorfen (240 mg/L), was also found to degrade the herbicide via the same pathways; however in this study, the bacteria used oxyfluorfen as the sole source of carbon (Chakraborty et al. 2002).

Mohamed et al. (2011) determined soil degradation depends on species and temperature by exposing microbial degraders to various oxyfluorfen concentrations (0, 96, 200, 400, 800, and 4000 mg ai/kg soil; 28°C and 40°C). These microbes degraded more oxyfluorfen at a higher temperature (40°C, 55.2–78.3%) than at a lower temperature (28°C, 17.5–36.6%) after 45 days. The authors measured oxyfluorfen degradation after 21 days of incubation in a mineral salt medium. Within the exposed soil, the authors identified ten bacterial and fungal isolates that displayed a degradation rate of 35.6–95.6%.

5 Toxicology

5.1 Mode of Action

Oxyfluorfen is a peroxidizing herbicide that achieves toxicity in plants via inhibition of the protoporphyrinogen oxidase (Protox) enzyme (Shaner 2014). The main target, Protox, is an enzyme of chlorophyll and heme biosynthesis (Herbicide Handbook 2014). Protox oxidizes protoporphyrinogen IX (Proto IX) to protoporphyrin IX (Proto IX); oxyfluorfen prevents this oxidation, thus Proto IX accumulates. Proto IX leaks out of the chloroplast into the cytoplasm, where it readily oxidizes into Proto IX. Once oxidized, Proto IX cannot go back into

the chloroplast due to its non-polar state, and partitions into the membranes (Jung and Kuk, 2007). In the cytoplasm, Proto IX absorbs light and produces triplet state Proto IX, which reacts with oxygen and forms singlet oxygen. Both triplet Proto IX and singlet oxygen extract hydrogen from unsaturated lipids in the cell and chloroplast membranes producing lipid radicals which in turn initiate chain reactions of lipid peroxidation (Herbicide Handbook 2014). Once exposed to light and molecular oxygen within the cytoplasm, Proto IX generates reactive singlet oxygen radicals, causing peroxidation of the cell membrane, eventually leading to necrosis and cell death (Jung and Kuk 2007).

Oxyfluorfen may also inhibit photosynthesis. The destruction of the chloroplast membranes from the inhibition of the Protox enzyme disrupts the photosynthetic mechanism of the plant. Sharma et al. (1989) discovered that oxyfluorfen damages chloroplast membranes by inhibiting photosystem II (PSII) and electron transport in plants. A dose-dependent experiment with 15-day-old rice plants confirmed that reduction in chlorophyll occurred with a parallel dose-dependent decline in PSII activity. Rice plants exposed to 1 mg/L oxyfluorfen showed a slight decline in chlorophyll content and PSII activity. However, when the rice plants were exposed to 3, 5, or 7 mg a.i./L, irreversible and permanent reductions in chlorophyll content (80% loss at 7000 µg a.i./L; 5 days post-treatment) and PSII activity (90% reduction at 7 mg a.i./L; 5-days post-treatment) were observed (Sharma et al. 1989).

5.2 *Terrestrial Plants*

Oxyfluorfen affects terrestrial plants when directly applied to the soil (pre-emergence) or sprayed over the plants (post-emergence). It causes general foliar necrosis within two days of exposure when applied post-emergence (Shaner 2014). As with other Protox inhibitors, the leaf appears to

be soaked with water and the leaf tissue turns a dark green color. Necrosis and desiccation of the affected tissue follows (Hess 2000). Other signs of phytotoxicity include weak chlorosis, deformation of leaf and vegetation tips, and inhibition of plant growth due to reduced and ineffective functioning leaf stomata (Anastasov 2010).

The US EPA (2002) reviewed two bioassay studies to identify the potential exposure to non-target species via pre-emergence or post-emergence application. Shoot length was used to assess effects of the pre-emergence application; and shoot weight was used to assess post-emergence application effects. During the 14-day study using emulsifiable concentrate formulation (71.5% a.i.), various concentrations of oxyfluorfen (0.0002914 to 1.793 kg a.i./ha) were tested on six dicotyledon plants (soybean, lettuce, carrot, tomato, cucumber, and cabbage) and four monocotyledon plants (corn, oat, ryegrass, and onion). In the pre-emergence test, cabbage, lettuce, onion, and ryegrass were the most sensitive to oxyfluorfen with calculated 25% effective concentration (EC_{25}) values of 0.002914, 0.003026, 0.004259, and 0.006501 kg a.i./ha, respectively. Additionally, carrot, corn, cucumber, oat, soybean, and tomato were the most sensitive in the post-emergence test with calculated EC_{25} values of 0.030264, 0.106486, 0.013451, and 0.000482 kg a.i./ha, respectively (US EPA 2002). Results of these tests show that oxyfluorfen is quite toxic to the exposed non-target terrestrial plants. In a greenhouse study conducted by Jusaitis (1993), post-emergence treatment using emulsifiable concentrate Goal® CT (24% a.i) was visually assessed on 18 species of native Australian plants. Oxyfluorfen was applied (1 kg a.i./ha) over the top of all 18 plants using a gas-pressurized backpack sprayer. After 5 days of application all of the treated plants had symptoms that included leaf spotting, degeneration of apical tissue, and severe necrosis of leaf tissue except for one plant. However, 20

days post-application seven species outgrew any phytotoxicity damages and 85 days post-application all species except for four plants had completely outgrown any phytotoxicity damages (Jusaitis 1993).

Crop species show considerable variation in their tolerance to oxyfluorfen. Comparative studies reported that a few crops including peanut, soybean, cotton (Matsumoto et al. 1994) and wheat (Chun et al. 2001) are relatively tolerant to oxyfluorfen. Researchers have attempted to genetically engineer more oxyfluorfen-tolerant crops by isolating and implanting Protox genes into plant genetic material. Choi et al. (1998) and Lee et al. (2000) used Protox genes from *Bacillus subtilis* to create transgenic tobacco and rice. Transgenic rice expressing Protox genes was further engineered from *Myxococcus xanthus* (Jung et al. 2004), *Arabidopsis thaliana* (Ha et al. 2003), and *Homo sapiens* (Lee et al. 2004); all showed higher levels of tolerance than the wild-type rice. Currently, oxyfluorfen transgenic crops are not commercially available.

5.3 Aquatic Plants

Like terrestrial plants, oxyfluorfen similarly affects aquatic plants through inhibition of photosynthesis and reduced growth (US EPA 2002). Amongst algal species, green algae are the most sensitive to oxyfluorfen. Rojickova-Padrtova and Marsalek (1999) conducted a study using the formulated product Goal® 2E (24% a.i.) to determine the toxicity of seven species of green and blue-green algae. Of the species tested, the most sensitive was a green algae (*Scenedesmus subspicatus*) that had a 72-hr EC₅₀ value of 0.676 µg a.i./L, whereas the most tolerant species was a blue-green algae (*Synechococcus leopoliensis*) that had a 72-hr EC₅₀ value of 49676.1 µg

a.i./L. The green algae, *S. capricornutum*, was reported as the most sensitive non-vascular species to oxyfluorfen with a 96-hr EC₅₀ value of 0.29 µg a.i./L; duckweed (*Lemna gibba*) was the most sensitive vascular species with a 14-d EC₅₀ value of 0.35 µg a.i./L (Table 2; US EPA 2002, 2009).

Geoffroy et al. (2003) studied the effect of oxyfluorfen exposure on green algae (*Scenedesmus obliquus*) at the cellular level, using 13 biomarkers to determine chlorophyll content, growth rate, photosynthesis, and antioxidant enzyme activity after being exposed for 12 and 48 hours to either 7.5 µg a.i./L, or 22.5 µg a.i./L concentrations of oxyfluorfen. The authors observed decreases in chlorophyll biosynthesis by 41% and 96%, respectively, to 7.5 and 22.5 µg a.i./L concentrations, and a decrease in cell growth by 50% after 48 hours of exposure to 22.5 µg a.i./L of oxyfluorfen. The study concluded that some photosynthetic and enzymatic biomarkers can be useful indicators of toxicity for non-target alga species and that analyzing photosynthetic biochemical processes may be a convenient tool for evaluating toxicity assessment of water quality (Geoffroy et al. 2003).

5.4 Fish & Aquatic Invertebrates

Oxyfluorfen is acutely and chronically toxic to a variety of aquatic species (US EPA 2002, 2009). Acutely, it is very highly toxic to freshwater and estuarine aquatic invertebrates such as waterflea (*Daphnia magna*) and grass shrimp (*Palaemonetes pugio*), and highly toxic to freshwater and estuarine aquatic fish such as bluegill sunfish (*Lepomis macrochirus*) and sheepshead minnow (*Cyprinodon variegatus*; Table 2; US EPA 2002, 2009). The lowest chronic toxicity values (NOAEC) were 1.3 µg a.i./L from fathead minnow (*Pimephales promelas*) and 13 µg a.i./L from *Daphnia magna* (Table 2).

Oxyfluorfen caused significant inhibition of brain acetylcholinesterase (AChE) activity in two fish species, Nile tilapia (*Oreochromis niloticus*) (52.7 – 81.3% of control) and mosquitofish (*Gambusia affinis*) (15.7 – 30.64% of control) after 15 days of exposure to oxyfluorfen (Goal® 2E emulsifiable concentrate 1000 µg a.i./L; Hassanein 2002). Inhibition of AChE is an important biomarker since its inhibition may lead to the accumulation of the neurotransmitter acetylcholine (ACh) at the neural synapse (El-Aleimy 1986). The inhibition of neurotransmission brought about by the accumulation of ACh can block activity in the respiratory center of the brain or in the neuromuscular junction of the respiratory system, leading to death (Soliman et al. 1995). This mechanism, in which oxyfluorfen induces neurotoxicity, is similar to the one observed by organophosphate pesticides (Hassanein, 2002). When fish have reduced AChE behavioral abnormalities may occur which include irregular, random, circular swimming movements, hyperexcitability, loss of equilibrium, sinking to the bottom, and inability to protect themselves from predation (Hassanein 2002).

5.5 Terrestrial Invertebrates

Oxyfluorfen is practically non-toxic to insects like honey bees ($LD_{50} > 100$ µg/bee, US EPA 2002) but studies have shown soil macro-organisms are adversely affected. In a slide-dip bioassay study, predatory mites (*Neoseiulus fallacis*) experienced a mortality of 85% after 24 hours and 96% after 48 hours exposure to the formulated product Goal® 2XL (23% a.i.) at a concentration of 1.856 mg/L at 21-24°C (Metzger & Pfeiffer 2002). Similarly, *Oribatida* mite and *Gamasina* soil mite species richness decreased by 50% and 33% , respectively, one year after pre-emergent application to willow tree plantings (0.1837 kg a.i./ha; Minor & Norton 2008). However, residue from a rye cover crop reduced the impact of oxyfluorfen on *Oribatida*

mites, most likely due to greater organic matter content for oxyfluorfen sorption and ideal conditions for microbial degradation. Thus, the effects of this herbicide on soil mites can be mitigated to an extent (Minor & Norton 2008).

When exposed to oxyfluorfen, both parasitoids and earthworms showed signs of decreased reproduction. Menezes et al. (2012) discovered that the formulated product Goal BR (24%) at a concentrations of 0.96 kg a.i./ha reduced parasitism and cocoon emergence of the parasitoid *Palmistichus elaeisis* (70-80% compared to the control) when exposed to sprayed pupa of the potential host *Tenebrio molitor* (7.48 µg/ pupa). Moreover, the herbicide altered the sex ratio of emerging *P. elaeisis*, favoring males over females (Menezes et al. 2012). Tejada et al. (2016) studied the effects of earthworms (*Eisenia fetida*, *Lumbricus terrestris* and *Allolobophora molleri*) on the bioremediation of contaminated soils exposed to the formulated product Fenfen (24% a.i.) at a concentration of 0.96 kg a.i./ha . Measurements revealed reduced glutathione-S-transferase activity, number of cocoons, average weight per cocoon, number of hatchlings per cocoon, and earthworm weight for all three species. Thus, both parent and daughter generations were negatively affected (Tejada et al. 2016).

5.6 Birds

Dietary studies have shown that oxyfluorfen is practically non-toxic to game birds. Exposure of both the bobwhite quail (*Colinus virginianus*) and mallard duck (*Anas platyrhynchos*) resulted in a 5-d 50% lethal concentration (LC₅₀) endpoint value of >5000 mg a.i./ kg-bw (US EPA 2002). Another study conducted over 20 weeks with the same species revealed similar results (Anatra-Cordone et al. 2005). Hoffman et al. (1991) studied American Kestrel (*Falco sparverius*)

nestling response to dietary oral intubation of corn oil dosed with oxyfluorfen at 10, 50, 250, and 500 mg a.i./kg corn oil. After 10 days at 500 mg a.i./kg, no nestling mortalities occurred (Hoffman et al. 1991).

6 Summary

The nitrodiphenyl ether herbicide oxyfluorfen was introduced in 1976 by the Rohm and Haas Company and has seen use in pre- and post- emergent control of broadleaf and grassy weeds in wine grapes, fruit orchards, vegetables, cotton, and other field crops. The herbicidal effect occurs by inhibiting protoporphyrinogen oxidase resulting in the accumulation of protoporphyrinogen IX, the generation of singlet oxygen under light conditions, and cell membrane damage.

Oxyfluorfen's low vapor pressure and Henry's law constant indicate volatilization is not a major route of environmental dissipation for the herbicide. Its high octanol-water partitioning coefficient indicates that it is resistant to leaching and not likely to appear in ground water. This is particularly true in soils of high organic matter and clay content. Oxyfluorfen contaminates surface water via runoff of eroded soil particles to which oxyfluorfen sorbed. Oxyfluorfen is mainly degraded in the environment by aqueous photolysis and microbial degradation.

The herbicide is toxic to various non-target species. Toxicity to birds is low however, toxicity to aquatic organisms, like fish and invertebrates, is high. Aquatic plants and algae are the most vulnerable non-target organisms due to common biochemical pathways among aquatic and terrestrial autotrophs. Therefore, as with all herbicides, care should be taken to reduce

environmental contamination and risk to non-target species when applying oxyfluorfen containing pesticides.

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References

Alister CA, Gomez PA, Rojas S, & Kogan M (2009) Pendimethalin and oxyfluorfen degradation under two irrigation conditions over four years application. *J Environ Sci Health B* 44(4):337-343.

Anastasov H (2010) Influence of oxyfluorfen on some anatomic indices in the leaves of Virginia tobacco plant (*Nicotiana Tabacum L.*). *Biotechnology & Biotechnological Equipment* 24(sup1): 33-35.

Anatra-Cordone M, King C, Klotzbach J, Durkin PR (2005) Oxyfluorfen - human health and ecological risk assessment- final report.

http://www.fs.fed.us/foresthealth/pesticide/pdfs/122205_Oxyfluorfen.pdf

CDPR (California Department of Pesticide Regulation). (2015) Top 100 Lists: The Top 100 Pesticides Used Statewide (All Sites Combined) in 2015. California Environmental Protection Agency, Sacramento, CA, p 1.

http://www.cdpr.ca.gov/docs/pur/pur15rep/top_100_ais_lbs_2015.pdf

Chakraborty SK, Bhattacharyya A, Chowdhury A (2002) Degradation of oxyfluorfen by *Azotobacter chroococcum* (beijerinck). *Bull Environ Contam Toxicol* 69(2):203-209.

- Choi KW, Han O, Lee HJ, Yun YC, Moon YH, Kim M, Kuk YI, Han SU, Guh JO (1998) Generation of resistance to the diphenyl ether herbicide, oxyfluorfen, via expression of the *Bacillus subtilis* protoporphyrinogen oxidase gene in transgenic tobacco plants. *Biosci Biotechnol Biochem* 62(3):558-560.
- Chun JC, Lee HJ, Lim SJ, Kim SE, Guh JO (2001) Comparative absorption, translocation, and metabolism of foliar-applied oxyfluorfen in wheat and barley. *Pest Biochem Physiol* 70:118-125.
- Coscollà C, Colin P, Yahyaoui A, Petrique O, Yusà V, Mellouki A, Pastor A (2010) Occurrence of currently used pesticides in ambient air of Centre Region (France). *Atmos Environ* 44(32):3915-3925.
- Deng X (2017) Surface water monitoring for pesticides in agricultural areas of California, 2016 Environmental Monitoring Branch. California Department of Pesticide Regulation. http://www.cdpr.ca.gov/docs/emon/pubs/ehapreps/deng_report_304.pdf
- Ensminger M (2016) Ambient and Mitigation Monitoring in Urban Areas in Northern California. Environmental Monitoring Branch. California Department of Pesticide Regulation. http://www.cdpr.ca.gov/docs/emon/pubs/ehapreps/report_269_ensminger_FY14_15.pdf
- Geoffroy L, Dewez D, Vernet G, Popovic R (2003) Oxyfluorfen toxic effect on *S. obliquus* evaluated by different photosynthetic and enzymatic biomarkers. *Arch Environ Contam Toxicol* 45:445-452.
- Ha SB, Lee SB, Lee Y, Yang K, Lee N, Jang SM, Chung JS, Jung S, Kim YS, Wi SG, Back K (2003) The plastidic *Arabidopsis* protoporphyrinogen IX oxidase gene, with or without the transit sequence, confers resistance to the diphenyl ether herbicide in rice. *Plant Cell Environ* 27:79-88.
- Hassanein HMA (2002) Toxicological effects of the herbicide oxyfluorfen on acetylcholinesterase in two fish species: *Oreochromis niloticus* and *Gambusia affinis*. *J Environ Sci Health A* 37(4):521-527.
- Hess FD (2000) Light-dependent herbicides: an overview. *Weed Sci* 48:160-170.
- Hoffman DJ, Spann JW, LeCaptain LJ (1991) Developmental toxicity of diphenyl ether herbicides in nesting American kestrels. *J Toxicol Environ Health* 34(3):323-336.
- Janaki P, Sathya Priya R, Chinnusamy C (2013) Field dissipation of oxyfluorfen in onion and its dynamics in soil under Indian tropical conditions. *J Environ Sci Health B* 48(11):941-947.
- Jung S, Lee Y, Yang K, Lee SB, Jang SM, Ha SB, Back K (2004) Dual targeting of *Myxococcus xanthus* protoporphyrinogen oxidase into chloroplasts and mitochondria and high level oxyfluorfen resistance. *Plant Cell Environ* 27:1436-1446.

- Jung H, Kuk Y I (2007) Resistance mechanisms in protoporphyrinogen oxidase (PROTOX) inhibitor-resistant transgenic rice. *Journal of Plant Biology* 50(5): 586-594.
- Jusaitis M (1993) Safety and efficacy of pre-emergent herbicides in container-grown Australian plants. *Plant Protection Quarterly* 8:127–130.
- Keum YS, Lee YJ, Kim JH (2008) Metabolism of nitrodiphenyl ether herbicides by dioxin-degrading bacterium *Sphingomonas wittichii* RW1. *J Agric Food Chem* 56(19):9146-9151.
- Lee HJ, Lee SB, Chung JS, Han SU, Han O, Guh JO, Jeon JS, An G, Back K (2000) Transgenic rice plants expressing a *Bacillus subtilis* protoporphyrinogen oxidase gene are resistant to diphenyl ether herbicide oxyfluorfen. *Plant Cell Physiol* 41(6):743-749.
- Lee Y, Jung S, Back K (2004) Expression of human protoporphyrinogen oxidase in transgenic rice induces both a photodynamic response and oxyfluorfen resistance. *Pest Biochem Physiol* 80(2):65-74.
- Mantzou N, Karakitsou A, Hela D, Patakioutas G, Leneti E, Konstantinou I (2014) Persistence of oxyfluorfen in soil, runoff water, sediment and plants of a sunflower cultivation. *Sci Total Environ* 472:767-777.
- Matsumoto H, Lee JJ, Ishizuka K (1994) Variation in crop response to protoporphyrinogen oxidase inhibitors. *Am Chem Soc Symp Ser* 559:120-132.
- Menezes CWG, Soares MA, Santos JB, Assis Junior SL, Fonseca AJ, Zanuncio JC (2012) Reproductive and toxicological impacts of herbicides in *Eucalyptus* culture in Brazil on the parasitoid *Palmistichus elaeisis* (Hymenoptera: Eulophidae). *Weed Res* 52:520-525.
- Metzger JA, Pfeiffer DG (2002) Topical toxicity of pesticides used in Virginia vineyards to the predatory mite, *Neoseiulus fallacis* (Garman). *J Entomol Sci* 37(4):329-337.
- Minor MA, Norton RA (2008) Effects of weed and erosion control on communities of soil mites (Oribatida and Gamasina) in short-rotation willow plantings in central New York. *Can J For Res* 38:1061-1070.
- Mohamed AT, El Hussein AA, El Siddig MA, Osman AG (2011) Degradation of oxyfluorfen herbicide by soil microorganisms biodegradation of herbicides. *Biotechnology* 10(3):274-279.
- Rojíčková R, Maršálek B (1999) Selection and sensitivity comparisons of algal species for toxicity testing. *Chemosphere* 38(14):3329-3338.
- Scrano L, Bufo SA, D' Auria M, Emmelin C (1999) Photochemical behavior of oxyfluorfen: a diphenyl-ether herbicide. *J Photochem Photobiol A: Chem* 129(1):65-70.
- Shaner DL (2014) *Herbicide Handbook*, 10th ed. Lawrence, KS, USA, pp 335-336.

Soliman FM, El-Elaimy IA, Hamada HMA (1995) Malathion toxicity to *Gambusia affinis* and its effect on brain acetylcholinesterase activity. J Agric Res 40: 227-242.

Sharma D, Bhardwaj R, Maheshwari V, Nagar S (1989) Oxyfluorfen binds to and inhibits photochemistry of photosystem II. Current Science 63(1): 1334-1336.

Tejada M, Gómez I, Franco-Andreu L, Benitez C (2016) Role of different earthworms in a soil polluted with oxyfluorfen herbicide. Short-time response on soil biochemical properties. Ecol Eng 86:39-44.

Thomas JP, Bejjani A, Nsouli B, Gardon A, Chovelon JM (2009) In situ studies of pesticides photodegradation on soils using PD-TOFMS technique: Application to norflurazon and oxyfluorfen. Int J Mass Spectrom 279(2):59-68.

Tomlin, CDS (2003) The pesticide manual: a world compendium, 13th ed. Hampshire, UK, pp 738-739.

US EPA (2001a) Oxyfluorfen: toxicology chapter for RED. Pesticides and toxic substances. US Environmental Protection Agency Office of Prevention. <https://www.fluoridealert.org/wp-content/pesticides/oxyfluorfen.toxicology.2001.pdf>

US EPA (2001b) Revised Environmental Fate and Effects Division preliminary risk assessment for oxyfluorfen reregistration eligibility decision document. <http://fluoridealert.org/wp-content/pesticides/oxyfluorfen.enveffects.2001.pdf>

US EPA (2002) Reregistration eligibility decision (RED) for Oxyfluorfen Case No. 2490. Pesticides and toxic substances. US Environmental Protection Agency Office of Prevention. https://archive.epa.gov/pesticides/reregistration/web/pdf/oxyfluorfen_red.pdf

US EPA (2009) Risks of oxyfluorfen use to the federally threatened California red-legged frog (*Rana aurora draytonii*). <https://www.regulations.gov/document?D=EPA-HQ-OPP-2014-0778-0006>

US EPA (2017) Aquatic life benchmarks for pesticide registration. <https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/aquatic-life-benchmarks-pesticide-registration>

Wofford P, Segawa R, Schreider J, Federighi V, Neal R, Brattesani M (2014) Community air monitoring for pesticides. Part 3: using health-based screening levels to evaluate results collected for a year. Environ Monit Assess 186(3):1355-1370.

Yen JH, Sheu WS, Wang YS (2003) Dissipation of the herbicide oxyfluorfen in subtropical soils and its potential to contaminate groundwater. Ecotoxicol Environ Saf 54(2):151-156.

Ying GG, Williams B (1999) The degradation of oxadiazon and oxyfluorfen by photolysis. J Environ Sci Health B 34(4):549-567.

Ying GG, Williams B (2000) Dissipation of herbicides in soil and grapes in a South Australian vineyard. *Agric Ecosyst Environ*, 78(3):283-289.

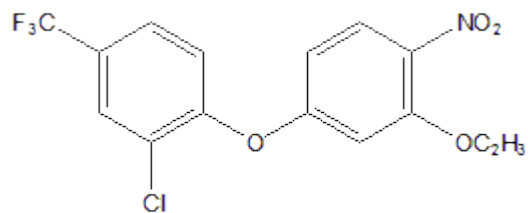


Figure 1 Structure of oxyfluorfen

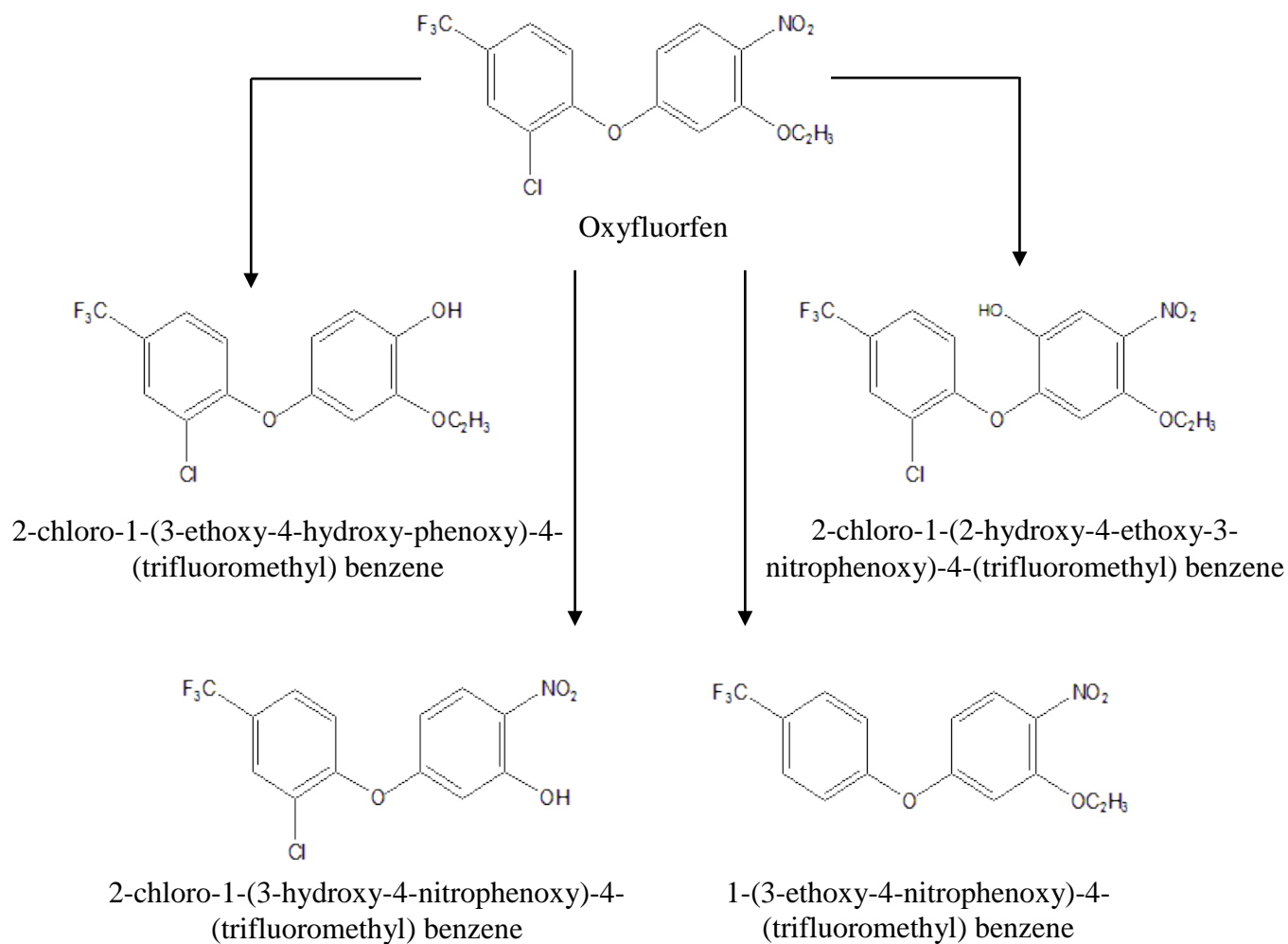


Figure 2 Major oxyfluorfen degradates from photolysis in acetonitrile solvent (adapted from Scrano et al. 1999).

Table 1 Physicochemical properties of Oxyfluorfen

Chemical Abstract Service registry number (CAS#) ^a	42874-03-3
Molecular weight (g/mol) ^a	361.72
Density at 73°C (g/mL) ^a	1.35
Melting point (°C) ^a	65-84
Octanol-water partition coefficient (log K _{ow}) ^b	4.46
Organic carbon normalized partition coefficient (K _{OC}) ^b (mL/g)	10 ⁵
Vapor pressure at 25°C (mPa) ^b	0.0267
Henry's law constant (Pa m ³ mol ⁻¹) ^d	0.0833
<i>Solubility at 25°C (mg/L)^d</i>	
Water	
<i>Solubility at 25°C (g/kg)^e</i>	
Acetone	0.116
Cyclohexanone	61.5
Isophorone 61.5	72.5
Dimethylformamide	>50
Chloroform	50-55
Mesityl oxide	40-50

^a) Data from US EPA (2001b), ^b) Data from Shaner (2014), ^c) Data from Coscollà et al (2010), ^d) Data from US EPA (2002), ^e) Data from Tomlin (2003)

Table 2 Toxicity values for aquatic organisms^{a)}

Taxa	Aquatic Organism	Scientific name	Test type	Concentration (µg/L)
Fish				
	Bluegill Sunfish	<i>Lepomis macrochirus</i>	96-hr LC ₅₀	200
	Rainbow Trout	<i>Oncorhynchus mykiss</i>	96-hr LC ₅₀	250
	Sheepshead Minnow	<i>Cyprinodon variegatus</i>	96-hr LC ₅₀	>170
	Fathead Minnow	<i>Pimephales promelas</i>	NOAEC	1.3
Invertebrates				
	Waterflea	<i>Daphnia magna</i>	48-hr EC ₅₀ NOAEC	80 13
	Grass Shrimp	<i>Palaemonetes pugio</i>	96-hr LC ₅₀	32
Non-vascular plants				
	Green Algae	<i>Selenastrum capricornutum</i>	96-hr EC ₅₀	0.29
Vascular plants				
	Duckweed	<i>Lemna gibba</i>	14-d EC ₅₀	0.35

^{a)}Data from US EPA (2002, 2009).