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Evolution of Toxicity upon Hydrolysis of Fenoxaprop-*p*-ethyl

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Hydrolysis of fenoxaprop-*p*-ethyl (FE), a widely used herbicide, was studied in aqueous buffer solutions at pH ranging from 4.0 to 10.0. The degradation kinetics, strongly dependent on pH values, followed first-order kinetics. FE was relatively stable in neutral media, whereas it degraded rapidly with decreasing or increasing pH. In acidic conditions (pH = 4, 5), the benzoxazolyl-oxy-phenyl ether linkage of FE was cleaved to form ethyl 2-(4-hydroxyphenoxy)propanoate (EHPP) and 6-chloro-2,3-dihydrobenzoxazol-2-one (CDHB). While in basic conditions (pH = 8, 9, 10), herbicidal activity fenoxaprop-*p* (FA) was formed via breakdown of the ester bond of the herbicide. Both the two pathways were concurrent in neutral conditions (pH = 6, 7). Toxicity studies on *Daphnia magna* showed that FE was most toxic to *D. magna* with 48 h EC_{50} of 14.3 μmol/L, followed by FA (43.8 μmol/L), CDHB (49.8 μmol/L), and EHPP (333.1 μmol/L). Mode of toxic action analysis indicated that EHPP exhibited toxicity via polar narcosis, whereas CDHB belonged to reactive acing compound. The mixture toxicity of CDHB and EHPP was nonadditive and can be predicted by a response addition model. Therefore, the evaluation of overall FE toxicity to *D. magna* in the aquatic systems needs to consider the degradation of FE.

KEYWORDS: Fenoxaprop-*p*-ethyl; hydrolysis; *Daphnia magna*; toxicity

INTRODUCTION

Once released into the environment, pesticides will be transformed by physical, chemical, and biological processes, which may generate new chemicals (*1*). The transformation products would also possess the potential of adverse impacts on the ecosystem (*2*, *3*). Aquatic organisms are therefore exposed not only to individual pesticides but also to their metabolites and mixtures of them. Lack of toxicity data and information about modes of toxic action for transformation products makes it difficult to assess whether the transformation processes can result in more risks (*4*). Consequently toxicological effects of pesticide products are a matter of great concern owing to the fact that a large number of pesticides are in commercial use.

Fenoxaprop-*p*-ethyl [(+)-ethyl 2-[4-[(6-chloro-2-benzoxazolyl)-oxy]phenoxy]propanoate] (FE) is an aryloxyphenoxypropionate herbicide for selective control of various annual and perennial grass weeds in rice, rye, and soybeans (*5*). FE controls weeds by inhibiting acetyl-CoA carboxylase (ACCase) that was found both in plant chloroplasts and in mammalian livers (*6*−*8*). Liver toxicity of fenoxaprop-ethyl has been observed in long-term studies for mice (*9*). Fenoxaprop-ethyl was also believed to have reproductive toxicity and had a severe effect on motility patterns of porcine sperm (*10*, *11*), which together with the liver toxicity makes it impossible to underestimate the risk of the compound to human beings.

Generally, aryloxyphenoxypropionate herbicides are applied as ester forms because they are more rapidly absorbed by plants than the acid formulations (*12*). Hydrolysis can be a main degradation pathway for this class of herbicides since hydrolysis of esters is catalyzed by acid or base. The purpose of this study was to elucidate the hydrolysis behavior of FE in a wide range of pH levels, and assess the acute toxicity of the herbicide and its hydrolytic products using *Daphnia magna*. Binary mixture toxicity of the hydrolysis products was also evaluated to better understand the real situation in the aquatic environment.

MATERIALS AND METHODS

Chemicals. Fenoxaprop-*p*-ethyl (97%) was supplied by Ulrodragon Co. (Hangzhou, China). Fenoxaprop-*p* (FA) (97%), ethyl 2-(4-hydroxyphenoxy) propanoate (EHPP) (98%), and 6-chloro-2,3-dihydrobenzoxazol-2-one (CDHB) (97%) were purchased from Sigma-Aldrich, Yongnuo Pharma, Ltd. (Nanjing, China), and Tianchen Chemical Factory (Huai'an, China), respectively. All were used as received. All solvents used were HPLC grade.

Hydrolysis of FE. The stability of FE in 50 mL buffer solutions was determined at pH ranging from 4.0 to 10.0 (±0.1). Buffer solution at pH 4.0 was prepared by combining appropriate volumes of 0.1 M citric acid and 0.2 M disodium hydrogen phosphate. Solutions of pH 5.1, 6.0, 7.0, and 8.0 were prepared from 0.067 M phosphate buffer. Solutions at pH 9.0 and 10.0 were prepared from 0.1 M sodium hydroxide, boric acid, and potassium chloride solution. All the solutions

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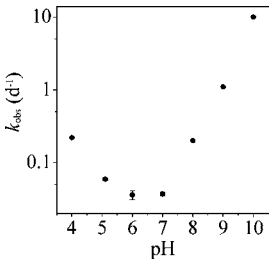

Figure 1. Variation of k_{obs} of fenoxaprop-*p*-ethyl with pH at 30 °C.

were prepared using autoclaved high-purity water. Solubility limitations of FE required use of cosolvent acetonitrile (5%) to achieve an initial concentration of 5.5 μmol/L. All reactions were carried out in glassware, which were baked in a muffle furnace (400 °C) and then sterilized before use. Samples were maintained at 30 °C in the dark. Each experiment was run in duplicate and terminated at the end of 45 days.

Analytical Procedure. Aliquots (0.5 mL) of the reaction solutions were periodically removed at designed intervals, and analyzed immediately by an Agilent 1100 high-performance liquid chromatography (HPLC) equipped with a vacuum degasser, a quaternary pump, an autosampler, and a diode array detector. A Hypersil C_{18} analytical column (5.0 μm, 4.6 × 250 mm) eluting with acetonitrile plus water (70 + 30 by volume) at a flow rate of 1.0 mL/min was used in the analysis. The injection volume was 10 μL, and the detection wavelength was 240 nm. Hydrolysis metabolites were identified by comparing the HPLC retention times of the analytes with the authentic standards and by gas chromatography/mass spectrometry (GC-MS, Agilent 6890N/5975 MSD) simultaneously. The GC column was a HP-5 (30 m × 0.32 mm × 0.25 μm), and the temperature program was from 65 °C to 150 °C at a rate of 20 °C/min, then to 280 °C at 10 °C/min. The injection volume was 1 μL with helium as a carrier gas.

Toxicity Test. *D. magna* has been cultured in our laboratory for 5 years. Daphnids were cultured in 1 L of medium at a density of 40 animals per beaker and fed with *Chlorella pyrenoidosa* at a density of ca. 10^5 cells/mL. The medium was renewed three times a week.

The 48 h acute toxicity was determined according to the standard testing procedure of OECD (*13*). Each toxicity test consisted of 5 concentration treatments and a control without chemicals. The test was performed in beakers each containing 100 mL of test solution and ten neonates (≤24 h old) at 20 ± 1 °C under a 14 h light and 10 h dark photoperiod. The compounds were poorly water-soluble except for CDHB, so dimethyl sulfoxide (DMSO) was used as solubilizing agents with the final concentration not exceeding 0.1% (v/v). Control experiments with equivalent volumes of DMSO exhibited no inhibitory effects on the assay species, and experiments performed in *D. magna*-free blank solutions showed negligible loss of all the compounds. Immobilization of *D. magna* was recorded at 48 h if no movement occurred after gentle stirring of the test solutions within 15 s. The 48 h median effective concentration (EC_{50}) and 95% confidence limits (CL) were calculated using Probit analysis (US EPA, 1993). The 24 h EC_{50} of the reference compound $K_2Cr_2O_7$ varied between 0.7 and 1.1 mg/L and met the requirements of the OECD guideline (*13*). All the assays were repeated in triplicate.

Additive toxicity was investigated in combinations of CDHB and EHPP. Mixture exposures were similar in design to the toxicity assay of the individual compounds. Two different mixture ratios were chosen: for one mixture, the concentrations of chemicals were 1:1 of their EC_{50}, and for the other, 1:1 of their molar ratio. Concentration−response relationships for mixtures were determined by keeping the ratio as constant and changing the total mixture concentrations (*14*).

RESULTS AND DISCUSSION

Hydrolysis of FE. The hydrolysis of FE is pH dependent (**Figure 1**) and follows pseudo-first-order kinetics. FE degradation rates reach a minimum at pH 6.0, and increase with decreasing or increasing pH. For example, at pH 4.0 and 10.0 (half-lives are 3.2 and 0.07 day, respectively), FE degradation is ∼6-fold and ∼270-fold faster than at pH 6.0 (half-life is 19 days), respectively. Clearly, in the studied pH range FE exhibits characteristics of acid and, especially, alkaline-catalyzed hydrolysis. Similar results were reported by Toole et al. (*15*), who found that hydrolysis of FE was slow at pH 6.1 and 7.4, but was fast at pH 9.1.

Hydrolysis Metabolites. For hydrolysis of FE at pH 4.0 and 5.1, HPLC analysis showed the appearance of two peaks prior to FE. According to GC−MS analysis, the first peak showed an accurate *m*/*z* of 210, which was in good accordance with the retention time and mass spectrum of a pure EHPP standard. The second one yielded an accurate *m*/*z* of 169, which gave the best-fit formula of $C_7H_4NO_2Cl$. This formula is consistent with the formation of CDHB. These results reveal that the two products derive from the breakdown of the benzoxazolyl-oxyphenyl ether linkage of FE. Zablotowicz (*16*) also detected CDHB formation during 22 h hydrolysis of FE at pH < 4.6, but they did not ascertain the formation of EHPP.

At pH = 10.0, FE showed a total loss within 10 h, corresponded to the appearance of FA. The base-catalyzed hydrolysis of FE proceeds by the direct nucleophilic addition of hydroxide ion (OH^-) to the carbonyl group. Actually, de-esterification of FE was observed in the pH range of 6−10, but the production of FA was higher at pH 10.0 than 6.0. All three products, FA, CDHB, and EHPP, were observed for the degradation of FE at pH = 6.0. These findings support the different hydrolysis mechanisms of FE at different pH levels (*17*), which was summarized in **Figure 2**.

Single Substance Toxicity. The 48 h EC_{50} of FE and the metabolites acting singly to *D. magna* are summarized in **Table 1**. FE is more toxic to *D. magna* than the three hydrolysis products. Toxicity reduction of the hydrolysis products is partly linked to their lower hydrophobicity than the parent herbicide as described by octanol/water partition coefficients (K_{ow}). However, as shown in **Figure 3**, log EC_{50} of CDHB was underestimated by log K_{ow}, which may imply a different mode of toxic action. The enhanced toxicity of CDHB is associated with the electron-donating effect of the nitrogen atom (*18*), which is relatively electron-deficient because the lone electron pair on nitrogen is delocalized to both the carbonyl fragment and the aromatic ring (*18*). The electron-deficient effect is reinforced by the electron-acceptor property of the chlorine substituent at C_6 position. The electrophilic property of the nitrogen atom can lead to irreversible formation of covalent bonds with nucleophilic entities in biological molecules, such as proteins and DNA (*19*). Additionally, CDHB has antifungal activity and can inhibit the growth of the bacteria *Staphylococcus aureus*, *Escherichia coli*, and the fungi *Candida albicans* (*20*). CDHB also belongs to benzoxazolinone derivatives that have allelopathic activity.

Among FE and its hydrolysis products, EHPP shows the least toxicity to *D. magna*. However, both hydrogen bond donor (O−H) and acceptor (C═O) exist in its chemical structure. Besides, EHPP, with a log K_{ow} less than 3, shows weakly acidic character due to the −OH group. All of these characteristics are critical for polar narcosis (*22*, *23*). As for FA, it still has herbicidal activity, and studies with chloroplasts isolated from grass species showed that fenoxaprop was about 100 times more powerful than fenoxaprop-ethyl in inhibiting ACCase (*24*). However, in this study the acute toxicity of FA on *D. magna* is 3 times less toxic than that of FE. Thus, the mode of toxic action of the toxicophore may not be relevant for different test species, and biotests with nontarget organisms are essential for risk assessment (*4*).

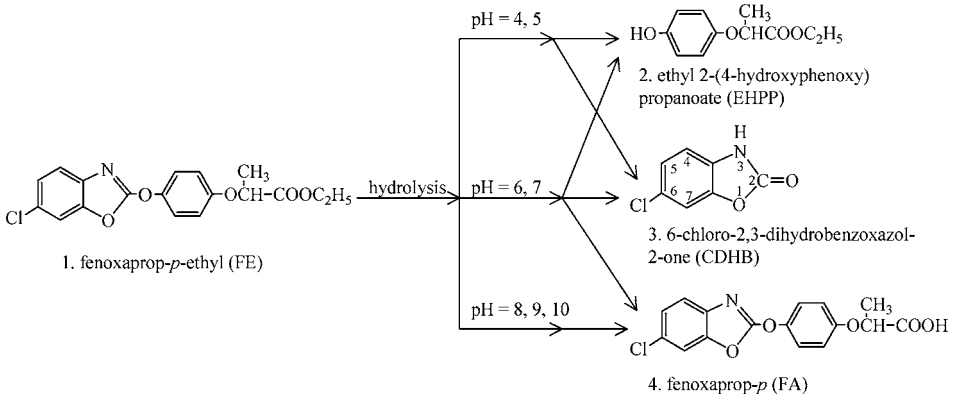

Figure 2. Hydrolysis mechanism of fenoxaprop-*p*-ethyl in different buffer solutions.

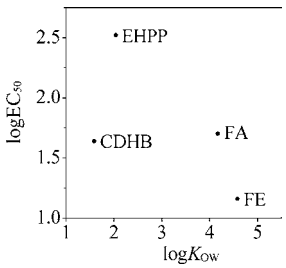

Figure 3. Plot of log EC_{50} versus log K_{ow}.

Table 1. log K_{ow} and 48 h EC_{50} to *D. magna* for the Tested Compounds

	48 h EC_{50}			
compounds	μmol/L	95% CL	mg/L	log K_{ow}[a]
FE[c]	14.3	11.6–19.1	5.2	4.58[b]
FA	43.8	38.8–49.3	14.6	4.17
CDHB	49.8	47.2–51.3	8.4	1.59
EHPP	333.1	277.6–396.3	70.0	2.04

[a] log K_{ow} was calculated by KOWWIN (U.S. EPA, 2000). [b] log K_{ow} of FE was from the experimental data (*21*). [c] 40% immobilization effect on *D. magna*.

Mixture Toxicity. CDHB and EHPP exist as mixtures owing to the nature of formations by cleavage of the ether linkage of FE. Except for hydrolysis of FE, studies on photodegradation of fenoxaprop-ethyl also revealed the formation of CDHB and EHPP (*15*). Due to temporal and spatial variability of the mixture concentrations, it is promising to predict the overall mixture toxicity from the single toxicity of the two degradates.

Concentration addition and response addition are two fundamental concepts for predictive assessments of mixture toxicity (*25*). The concept of concentration addition defined for the mixture of CDHB and EHPP can be expressed mathematically as

$$\mathrm{EC}\ x_{\mathrm{mix}} = \left(\frac{p_{\mathrm{CDHB}}}{\mathrm{EC}\ x_{\mathrm{CDHB}}} + \frac{p_{\mathrm{EHPP}}}{\mathrm{EC}\ x_{\mathrm{EHPP}}}\right)^{-1} \qquad (1)$$

where EC x_{mix} is the total concentration of the mixture provoking x effect, with x between 0 and 100%; EC x_{CDHB} and EC x_{EHPP} are respective concentrations of CDHB and EHPP that would alone cause the same effect x as observed for the mixture; and p_{CDHB} and p_{EHPP} are concentration fractions of CDHB and EHPP in the mixture, respectively. The basic idea of this concept is that the two components share a common mode of action to the exposed organism (*25*).

The alternative approach to predicting mixture toxicity of CDHB and EHPP is response addition:

$$E_{\mathrm{mix}} = 1 - (1 - E(c_{\mathrm{CDHB}}))(1 - E(c_{\mathrm{EHPP}})) \qquad (2)$$

where E_{mix} is the total effect of the mixture; $E(c_{CDHB})$ and $E(c_{EHPP})$ are the effects of CDHB and EHPP if applied singly in concentrations c_{CDHB} and c_{EHPP}, respectively. The concept of response addition assumes that the two mixture constituents each elicit their response through heterogeneous modes of action (*14*).

Results on the mixture toxicity of CDHB and EHPP are given in **Figure 4**, together with the corresponding fitted models. Irrespective of the mixture ratio and response level, the hypothesis of response addition yields accurate predictions of the mixture toxicity. On the 50% effect level, observed and predicted effect concentration did not differ by more than 2% for equal molar concentration mixture. In contrast, predictions based on the hypothesis of concentration addition tend to overestimate the mixture toxicity. The degree of overestimation varied with the response level and mixture ratio. These results support the notion that the mode of toxic action of CDHB is different from that of EHPP. Alternatively, it is possible to use

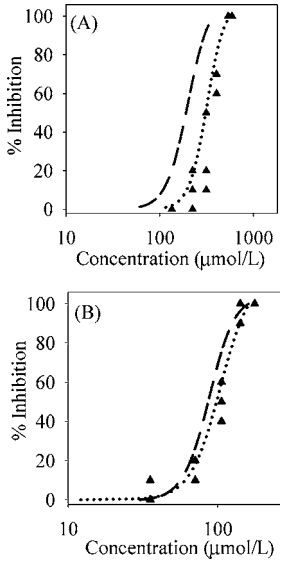

Figure 4. Observed and predicted *D. magna* mixture toxicity of CDHB and EHPP at (**A**) components mixed in the ratio of their individual EC_{50} values; (**B**) components mixed in equal molar concentration. (▲) Experimentally observed toxicity; (···) prediction according to response addition; (– – –) prediction according to concentration addition.

the mixture toxicity experiment to validate the modes of toxic action of pollutants (*26*).

ENVIRONMENTAL SIGNIFICANCE

FE hydrolysis followed two different mechanisms at different pH levels, yielding CDHB, EHPP, and FA. As a consequence of the degradation, the three products are less toxic than the parent herbicide. However, the product CDHB, which is also formed by photolysis, biodegradation, and transformation in soil of fenoxaprop-ethyl, belongs to reactive acting compound and can adversely affect the growth of water invertebrates, bacteria, fungi, even plants due to its allelopathic activity. Thus, hydrolysis of FE results in a product with a different and more potent mode of toxic action. Consequently there is a need to consider transformation products during the environmental risk assessment process.

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LITERATURE CITED

(1) Petit, V.; Cabridenc, R.; Swannell, R. P. J.; Sokhi, R. S. Review of strategies for modelling the environmental fate of pesticides discharged into riverine systems. *Environ. Int.* **1995**, *21*, 167−176.

(2) Sinclair, C. J.; Boxall, A. B. A. Assessing the ecotoxicity of pesticide transformation products. *Environ. Sci. Technol.* **2003**, *37*, 4617−4625.

(3) Escher, B. I.; Bramaz, N.; Richter, M.; Lienert, J. Comparative ecotoxicological hazard assessment of beta-blockers and their human metabolites using a mode-of-action-based test battery and a QSAR approach. *Environ. Sci. Technol.* **2006**, *40*, 7402−7408.

(4) Belfroid, A. C.; Van Drunen, M.; Beek, M. A.; Schrap, S. M.; Van Gestel, C. A. M.; Van Hattum, B. Relative risks of transformation products of pesticides for aquatic ecosystems. *Sci. Total. Environ.* **1998**, *222*, 167−183.

(5) Bieringer, H.; Horlein, G.; Langelluddeke, P.; Handte, R. HOE 33171: A new selective herbicide for the control of annual and perennial warm climate grass weeds in broadleaf crops. *Proc. Br. Crop Prot. Conf. Weeds* **1982**, *1*, 11−16.

(6) Tal, A.; Romano, M. L.; Stephenson, G. R.; Schwan, A. L.; Hall, J. C. Glutathione conjugation: A detoxification pathway for fenoxaprop-ethyl in barley, crabgrass, oat, and wheat. *Pestic. Biochem. Physiol.* **1993**, *46*, 190−199.

(7) Lee, W. M.; Elliott, J. E.; Brownsey, R. W. Inhibition of acetyl-CoA carboxylase isoforms by pyridoxal phosphate. *J. Biol. Chem.* **2005**, *280*, 41385−41843.

(8) Sasaki, Y.; Nagano, Y. Plant acetyl-CoA carboxylase: Structure, biosynthesis, regulation, and gene manipulation for plant breeding. *Biosci., Biotechnol., Biochem.* **2004**, *68*, 1175−1184.

(9) Leist, K. H. Toxicity test of HOE 33171 OH AT 203 in a 32-day study with SPF-mice, Hoechst Report # 336/80. **1980**, DPR 51910-005, #114443.

(10) State of California Environmental Protection Agency. Chemical known to the state to cause cancer or reproductive toxicity. **1999**. Available at Web site: www.oecd.org/dataoecd/5/37/2752836.pdf.

(11) Betancourt, M.; Reséndiz, A.; Fierro, E. Effect of two insecticides and two herbicides on the porcine sperm motility patterns using computer-assisted semen analysis (CASA) in vitro. *Reprod. Toxicol.* **2006**, *22*, 508−512.

(12) Bassett, M. V.; Pepich, B. V.; Munch, D. J. Role played by basic hydrolysis in the validity of acid herbicide data: Recommendations for future preparation of herbicide performance evaluation standards. *Environ. Sci. Technol.* **2000**, *34*, 1117−1121.

(13) OECD. 1998. *Daphnia magna* reproduction test. OECD TG 211, París, Francia.

(14) Faust, M.; Altenburger, R.; Backhaus, T.; Blanck, H.; Boedeker, W.; Gramatica, P.; Hamer, V.; Scholze, M.; Vighi, M.; Grimme, L. H. Joint algal toxicity of 16 dissimilarly acting chemicals is predictable by the concept of independent action. *Aquat. Sci.* **2003**, *63*, 43−63.

(15) Toole, A. P.; Crosby, D. G. Environmental persistence and fate of fenoxaprop-ethyl. *Environ. Toxicol. Chem.* **1989**, *8*, 1171−1176.

(16) Zablotowicz, R. M.; Hoagland, R. E.; Staddon, W. J.; Locke, M. A. Effects of pH on chemical stability and de-esterification of fenoxaprop-ethyl by purified enzymes, bacterial extracts, and soils. *J. Agric. Food Chem.* **2000**, *48*, 4711−4716.

(17) Pusino, A.; Petretto, S.; Gessa, C. Montmorillonite surface-catalyzed hydrolysis of fenoxaprop-ethyl. *J. Agric. Food Chem.* **1996**, *44*, 1150−1154.

(18) Bravo, H. R.; Lazo, W. Antialgal and antifungal activity of natural hydroxamic acids and related compounds. *J. Agric. Food Chem.* **1996**, *44*, 1569−1571.

(19) Harder, A.; Escher, B. I.; Landini, P.; Tobler, N. B.; Schwarzenbach, R. P. Evaluation of bioanalytical assays for toxicity assessment and mode of toxic action classification of reactive chemicals. *Environ. Sci. Technol.* **2003**, *37*, 4962−4970.

(20) Bravo, H. R.; Copaja, S. V.; Lazo, W. Antimicrobial activity of natural 2-benzoxazolinones and related derivatives. **1997**, *45*, 3255−3257.

(21) Available at Web site: http://www.apat.gov.it/site/_files/prodotti_fitosanitari/FENOXAPROP_P_ETHYL.pdf.

(22) Broderius, S. J.; Kahl, M. D.; Hoglund, M. D. Use of joint toxic response to define the primary mode of toxic action for diverse industrial organic chemicals. *Environ. Toxicol. Chem.* **1995**, *14*, 1591−1605.

(23) Verhaar, H. J. M.; Van Leeuwen, C. J.; Hermens, J. L. M. Classifying environmental pollutants. 1: Structure-activity relationships for prediction of aquatic toxicity. *Chemosphere* **1992**, *25*, 471−491.

(24) Yaacoby, T.; Hall, J. C.; Stephenson, G. R. Influence of fenchlorazole-ethyl on the metabolism of fenoxaprop-ethyl in wheat, barley, and crabgrass. *Pestic. Biochem. Physiol.* **1991**, *41*, 296−304.

(25) Altenburger, R.; Backhaus, T.; Boedeker, W.; Faust, M.; Scholze, M.; Grimme, L. H. Predictability of the toxicity of multiple chemical mixtures to *Vibrio Fischeri*: Mixtures composed of similarly acting chemicals. *Environ. Toxicol. Chem.* **2000**, *19*, 2341−2347.

(26) Altenburger, R.; Schmitt, H.; Schuurmann, G. Algal toxicity of nitrobenzenes: combined effect analysis as a pharmacological probe for similar modes of interaction. *Environ. Toxicol. Chem.* **2005**, *24*, 324−333.

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