WATER RESOURCES RESEARCH GRANT PROPOSAL

Title: Investigation of the abiotic reduction of the herbicides trifluralin and pendimethalin.

Keywords: Herbicides, Pendimethalin, Trifluralin, Reduction, Groundwater

Duration: March 1, 2000 to February 28, 2001

Federal Funds: Year 1: $19,840

Non-Federal (Matching) Funds: Year 1: $27,655

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Statement of Critical Regional or State Water Problems

Trifluralin (a,a,a-trifluoro-2,6-dinitro-*N,N*-dipropyl-*p*-toluidine) and pendimethalin (*N*-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzamine) are dinitroaniline herbicides used extensively throughout the midwestern United States. In 1992, approximately 20 million pounds of each of these compounds were used for agricultural purposes in the U.S. (*1*). In southwestern Minnesota counties, an average of 23 pounds of pendimethalin and 16 pounds of trifluralin were applied per square mile in 1992 (*1*). The widespread use of these herbicides has led to their detection as contaminants in groundwater, surface water, air, and precipitation (*2-4*) by the U.S. Geological Survey's National Water Quality Assessment (NAWQA) program. Trifluralin has also recently been found in oysters residing in Chesapeake Bay tributaries (*5*). These herbicides are applied by incorporation into the soil. Contamination of groundwater, therefore, may be caused by agricultural drainage (non-point sources). Point sources, such as spills or improper disposal, however, may also lead to groundwater contamination. Pendimethalin and trifluralin have frequently been detected in groundwater beneath herbicide manufacturing, loading, and mixing facilities (*2*).

The U.S. Environmental Protection Agency has classified these herbicides as persistent-bioaccumulative toxics (PBTs) and has recently lowered the Toxic Release Inventory (TRI) reporting threshold for these compounds (*6*). Trifluralin is also considered a possible human carcinogen (*7*). Even though the levels of these compounds detected are generally low, it is important to wholly understand the fate of trifluralin and

pendimethalin in groundwater in order to assess their environmental impacts and potential health threats. It is well known that these compounds sorb to soils (*8-11*). They may also sorb to aquifer sediments. This does not mean, however, that they would not be bioavailable and subject to bioaccumulation. For example, polychlorinated biphenyls (PCBs) strongly sorb to sediments, yet their bioaccumulation in food webs is well documented. Given the high usage rates of pendimethalin and trifluralin, the low levels detected in groundwater suggest that there may be additional loss mechanisms that are not yet understood. Although some work on biological degradation has been performed in soils (*12-14*), the potential role of abiotic reduction as a sink for the dinitroaniline herbicides has been largely ignored. If the nitro groups on these compounds are reduced to amines, such species could irreversibly bind to natural organic matter (*15*). Understanding of the potential loss mechanisms of trifluralin and pendimethalin in groundwater after they are introduced via spills, desorption from soils, or other sources will lead to better assessment of the potential threats these chemicals pose.

Statement of Expected Results or Benefits

The primary objective of this study is to evaluate the importance of reductive transformations as a sink for pendimethalin and trifluralin under a variety of conditions prevalent in groundwater. This research will use reductants thought to be representative of abiotic reductants found in natural systems: aqueous Fe(II), Fe(II) adsorbed onto a mineral surface (*e.g.*, the iron (hydr)oxide goethite), bisulfide, iron sulfide minerals (*e.g.*, pyrite), and hydroquinones (representing natural organic matter). If trifluralin and pendimethalin are rapidly degraded under reducing conditions, the impact of these parent compounds on groundwater supplies may be minimal. The anilines likely to be produced from the reduction of nitroaromatic compounds, however, are also of environmental concern. Identification of the products resulting from the reduction of trifluralin and pendimethalin and determination of the product fate will give additional information on the overall impact of pendimethalin and trifluralin on the environment.

The main benefit of this research will be a better understanding of the fate of trifluralin, pendimethalin, and their transformation products in the environment. To this point, the fate of these compounds under conditions typically found in groundwater has not been specifically analyzed. Thus, this study will fill an important knowledge gap. This study will also allow an analysis of NAWQA program data to determine the effect of redox conditions on herbicide fate in groundwater. For example, fast reduction rates of the dinitroanilines would be consistent with the low levels observed in groundwater. This project will benefit the people of Minnesota, and any region where trifluralin and pendimethalin are widely used, by providing a better understanding of the impact on groundwater supplies of agrochemicals considered to be toxic by the U.S. EPA.

Nature and Objectives of the Research

Trifluralin and pendimethalin are extensively used herbicides in Minnesota and the midwestern United States. These compounds have been detected in groundwater, surface water, and air. The main objective of the proposed study is to assess the importance of

abiotic chemical reduction as a loss mechanism for dinitroaniline herbicides. The fate of the resulting products will also be assessed. This research will lead to improved predictive models for the fate of these compounds in groundwater. Results will interpreted in the context of the data obtained by the USGS to see if the small scale laboratory results lend insight into the large scale observations of the NAWQA program.

Methods, Procedures, and Facilities

Phase I: Reduction of the parent compounds

The structures of the compounds of interest in this study, trifluralin and pendimethalin, are given in Figure 1. In Phase I, experiments will assess which of the potential reductants are capable of reducing these dinitroaniline herbicides. Inorganic reductants, such as Fe(II) (aqueous and adsorbed onto goethite), bisulfide, and pyrite will be studied. Also of interest is the potential role of natural organic matter (NOM) as a reductant. The structure of NOM is highly complex, so simple molecules thought to represent the functional groups responsible for the reductive capacity of NOM will be employed. The species to be used in this work are hydroquinone and reduced juglone (5-hydroxy-1,4-naphthoquinone). The sulfur adducts of quinones will also be evaluated as potential reductants and will be prepared by curing quinone or juglone in an anoxic bisulfide solution (*16, 17*).

Reduction experiments will be conducted anaerobically by deoxygenating all solutions and preparing the batch reactors (160 mL crimp-top serum bottles sealed with Teflon$^{®}$ faced septa) in an anaerobic chamber. Reactions will be conducted in the absence of headspace, to prevent losses via volatilization. To avoid potential competition from biological degradation, all glassware and solutions will be sterilized. Because the reduction potential of nitroaromatic compounds is pH dependent, experiments will be conducted at a number of pH values for each reductant to ascertain the influence of pH on reduction kinetics. The pH will be controlled using appropriate buffers. The dinitroaniline herbicides are photosensitive, so all reactors will be wrapped in aluminum foil to prevent losses due to photolysis. Reactors containing mineral surfaces will be mixed to prevent mass transfer limitation of reaction rates. Blanks without any reductant present will be used to quantify any disappearances due to hydrolysis or sorption.

For the homogeneous systems (aqueous Fe(II), hydroquinones, HS⁻), a large excess of reductant will be used so that the decay of the herbicide will display pseudo-first order kinetics. For the reductions that occur on a mineral surface (Fe(II)/goethite, pyrite), a large excess of reductant will also be present. Reaction rates on surfaces, however, are often limited by the availability of reactive sites. Thus, reactions may display first-, mixed- or zero-order kinetics. The initial concentrations of trifluralin and pendimethalin will be varied to test for intraspecies competitive effects, indicative of competition for reactive sites. The disappearance of trifluralin and pendimethalin will be monitored either by analysis of the aqueous solution with high performance liquid chromatography (HPLC) with a photodiode array detector (PDA) or by extracting the aqueous samples into an organic solvent followed by quantification via gas chromatography (GC) with

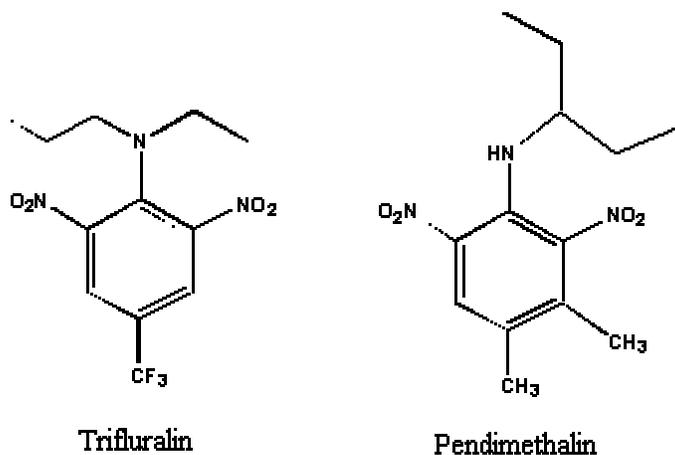electron capture detection (ECD). Phase I of the project will be completed within the first year.

Figure 1. Structures of the herbicides targeted in this study.

Phase II: Product identification

A number of products may be expected from the reduction of pendimethalin and trifluralin. The reduction of the nitro group follows the progression: nitro ($-NO_2$)→ nitroso ($-NO$) → hydroxylamine ($-NHOH$) → amine ($-NH_2$). Nitroso compounds are short lived and difficult to detect (*16, 18*). The compounds of interest contain two nitro groups, so expected products may include nitro/hydroxylamine, di-hydroxylamine, nitro/amine, hydroxylamine/amine, and di-amine species, as shown in Figure 2. Based on results with TNT, it is likely that the most important products will be the nitro/amine and di-amine species *(34, 35)*. Additional products may result when NOM-analogues are the reducing species (see Phase III). Whenever possible, potential intermediates and products will be purchased or synthesized to obtain authentic standards. The synthesis of a reduced form of pendimethalin containing one nitro group and one amine group has been reported (*14*). Di-amine species should be relatively easy to synthesize through use of a strong reductant, such as iron metal or tin/tin chloride, in an organic solvent. If authentic standards can not be obtained, degradation products will be identified by mass spectrometry (GC/MS or LC/MS; arrangements have been made for access to each of these instruments). Phase II will be completed over both years of the project.

Phase III: Binding of anilines to quinones

In the experiments where trifluralin and pendimethalin are reduced by hydroquinones or bisulfide-cured quinones, the resulting reduction products of the herbicides (anilines) may bind with the quinones produced from the oxidation of the NOM-analogues. When NOM-analogues are used as reductants, an attempt will be made to identify aniline-quinone addition products via LC/MS and/or nuclear magnetic resonance (NMR) (*19*).

The addition of the anilines to quinones may be slow relative to reduction rates of the parent compounds, and therefore not observable in the time scale of the reduction experiments. If so, the fate of the anilines will be investigated separately. Experiments will be conducted in which the disappearance of the mono- and di-aniline products are monitored in the presence of the oxidized NOM-analogues as well as soil humic acid using batch reactors similar to those described above. In this manner, the fate of the anilines resulting from the pendimethalin and trifluralin reduction can also be assessed. Again, if feasible, products will be identified using LC/MS and/or NMR. Phase III will be conducted in the second year of the project.
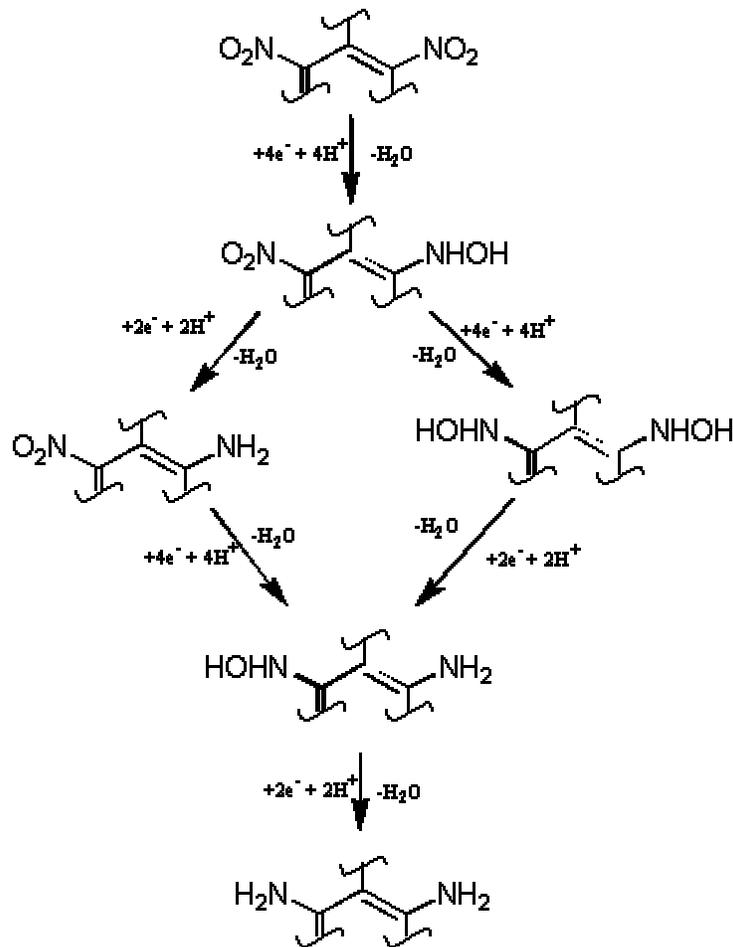

Figure 2. Simplified representation of potential intermediates from reduction of trifluralin and pendimethalin.

Related Research

There is a considerable body of work regarding the fate of trifluralin and pendimethalin. Much of this work, however, focuses on their behavior in a soil matrix or their degradation via photolysis. Sorption studies have determined that these compounds have large K_{OC} values and have demonstrated that considerable mass can partition into the soil

(8, 9, 11). Losses to the atmosphere after application due to volatilization have also been analyzed (20, 21). Given that pendimethalin and trifluralin are photosensitive, research has also been conducted to determine their fate when exposed to light. One product of trifluralin photodegradation is formed via dealkylation of the tertiary amine (12, 22). Subsequent reaction may lead to cyclization to form benzimidazoles (22). Photolysis appears to be strongly inhibited, however, when trifluralin is sorbed onto clay particles (22). Pendimethalin forms a variety of products resulting from dealkylation, reduction, and cyclization when exposed to ultra-violet light alone (23, 24) or in the presence of titanium dioxide (25, 26).

Degradation in soil seems to occur under both aerobic and anaerobic conditions. Some studies focused on disappearance rates, without attributing losses to a specific reaction mechanism (24, 27-29). Products of the degradation of pendimethalin (24) and trifluralin (28) , although not identified, appeared to be more polar than the parent herbicides, as they were more readily leached from the soils. Studies focused on the biodegradation of dinitroaniline herbicides in soils have also been performed. Aerobic biodegradation occurs via dealkylation of the tertiary amine group followed by reduction of the nitro group (13, 14). Under anaerobic conditions, sequential reduction of the nitro groups dominates (12-14). Results indicate that biotic degradation of trifluralin is accelerated as soil conditions become more reducing (13).

The disappearance rates and mechanisms found to occur in soils, however, may not be representative of those in groundwater. The abiotic reactions of substituted nitrobenzenes under reducing conditions have been actively studied by a number of researchers for several years. The reductants studied include hydroquinones and iron porphyrins (30), natural organic matter (16), Fe(II) adsorbed onto mineral surfaces (18), and bisulfide (31). Recent research has shown that sulfur adducts of NOM-analogues can reduce organic pollutants as well (17). For each of the reductants, the reaction of the nitrobenzenes proceeded through a hydroxylamine intermediate with an aniline being the final product. Much of this work, however, was conducted using compounds with a single nitro group, along with one additional electron withdrawing or electron donating substituent in the *ortho-*, *meta-*, or *para-* position. This systematic approach allowed relationships between reaction rate and reduction potential to be obtained (16, 18, 32, 33).

Only recently has such work been extended to study the potential role of naturally occurring reductants on the fate of polynitroaromatic groundwater contaminants, such as the explosive 2,4,6-trinitrotoluene (TNT). Hofstetter *et al.* (34) reported that TNT was completely reduced to the triaminotoluene by Fe(II) adsorbed onto iron (hydr)oxide minerals, and less efficiently by hydroquinone moieties in the presence of bisulfide. The work of Elovitz and Weber (35) revealed that sediments can partially reduce TNT and the resulting amines may bind to the sediments. Given that polynitroaromatic explosives are transformed by the reductants listed above, it is expected that similar reactions may also be important for trifluralin and pendimethalin. Electrochemical studies determined the reduction potential of trifluralin is -0.19 V (vs. SHE) at pH 5.1 and –0.3 V at pH 7.4 (36). These values are in a range that suggest that the naturally occurring reductants proposed for use in this study may be strong enough to make the overall reaction

thermodynamically favorable. In support of this notion, recent work has demonstrated that iron sulfide added to soils leads to rapid removal of trifluralin (*37*). No reaction products were isolated, but it was determined that an abiotic reaction (presumably reduction) was responsible for the observed trifluralin disappearance.

Anilines are also of environmental concern. It is important, therefore, to study the fate of the products formed via reduction of pendimethalin and trifluralin. It has been demonstrated previously that anilines will react with quinones by two mechanisms (*19, 38, 39*). There is a fast reversible reaction to form imines and also a slow irreversible 1,4 nucleophilic addition to the quinone ring. Anilines have also been found to bind soil organic matter (*40, 41*). The work of Weber *et al.* (*15*) has shown that the reaction of aniline with various humic and fulvic substance occurs via processes similar to that observed for the quinone NOM-analogues.

Trifluralin and pendimethalin are widely used herbicides that have the potential to contaminate groundwater supplies. This work will lend insight into the fate of these chemicals and their daughter products, leading to a better understanding of potential threats caused by the use of these compounds.

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