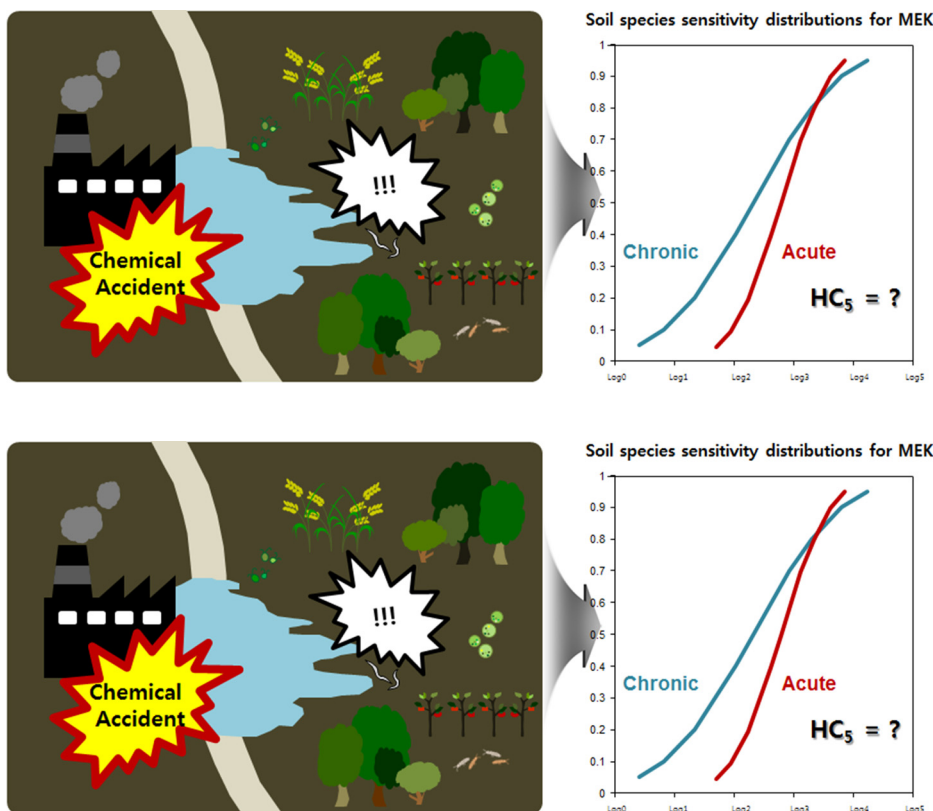# Ecological hazard assessment of methyl ethyl ketone using the species sensitivity distribution approach in a soil ecosystem

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GRAPHICAL ABSTRACT

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ABSTRACT

Methyl ethyl ketone (MEK) is a common and widely used industrial solvent. However, few studies have investigated its toxicity, or its effects as a contaminant in soil ecosystems. In this study, acute and chronic toxicity data for MEK were generated, and ecological risk based on a species sensitivity distribution was assessed. Seven soil organisms from six taxonomic groups were used for acute toxicity tests and five soil organisms from four

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Soil ecosystem

taxonomic groups were used for chronic toxicity tests. Acute and chronic soil HC_5 (hazardous concentration for 5% of species) values for MEK were estimated as 53.04 and 2.593 mg MEK/kg dry soil, respectively. This is the first study to conduct battery testing for MEK; it specifies hazardous concentrations, warns of the need for accident preparedness, and points to serious potential hazards of MEK at various levels of the soil ecosystem which can translate into greater environmental damage with implications for human health. The specific sensitivity levels determined may serve as a benchmark for establishing soil standards and strategies for ecosystem protection in the face of accidental contamination.

1. Introduction

Considerable amounts of chemicals are routinely used in industrial applications; however, their toxicities, and effects on the environment are poorly understood. There have been several chemical accidents in Korea in recent years (2012–2014) and many people have died or been injured by explosions or chemicals [1–5]. These accidents have highlighted the necessity for improved chemical safety, and led to the Ministry of the Environment's revision of Korea's Toxic Chemicals Control Act (TCCA) [1,6]. There are currently 97 accident preparedness substances recognized in the TCCA, including organic compounds, acid materials, volatile materials, toxic substances, and others.

MEK is one of these substances, and its use carries a substantial risk of chemical accidents. In October 2000, a tanker (*Ievoli Sun*) containing 1000 tons of MEK and other substances sank in the English Channel [7], and in 2002, a ship (*Bow Eagle*) carrying 1050 tons of MEK sank off the coast of Sein island, France [8]. In Korea, MEK has been associated with several chemical accidents, and in 2015 ranked fourth of all industrial chemicals emitted. MEK (or 2-butanone) is one of the most common volatile organic compounds in the ketone group, and is widely used as a solvent in paints and varnishes [9–12]. It is categorized as an R11 (highly flammable), R36 (irritating to the eyes), R66 (repeated exposure may cause skin dryness or cracking), and R67 (vapors may cause drowsiness and dizziness) toxic chemical by the European chemical Substances Information System (ESIS). In previous studies, MEK was shown to stimulate the mucous membranes of the eyes, nose, and throat at moderate concentrations ($\sim$ 200 ppmv) and caused paralysis of the central nervous system, anesthesia, and death at higher concentrations (300–600 ppmv) [13,14]. MEK can readily combine with other substances to make otherwise benign chemicals harmful [15–17]. Moreover, MEK can be easily absorbed through the skin and *via* inhalation [18].

Although several previous studies have focused on the toxicity of MEK to humans and other mammals, no study has directly assessed the ecotoxicity of MEK in a soil ecosystem, apart from a single evaluation of its toxicity to lettuce, *Lactuca sativa* [19]. Although several ecological risk assessments have been conducted for accident preparedness substances, including benzene, phenol, and toluene, in freshwater or marine ecosystems based on probabilistic approaches using species-sensitivity distributions (SSD) [20,21], no equivalent research has been carried out in a soil ecosystem. Given the increasing quantities of widely used chemicals and high numbers of chemical accidents, ecological risk assessments based on probabilistic approaches are needed to investigate the effects of accident preparedness substances on soil ecosystems.

In the present study, MEK was chosen as a model substance from the accident preparedness substances listed in the TCCA, and its toxicity to several species in the soil ecosystem was investigated. Seven species from six taxonomic groups, and including producers, consumers, and decomposers were selected to represent the soil ecosystem; additionally, the selected species had different body masses, exposure routes, and life cycles. Acute and chronic hazardous concentrations (HC) for MEK in the soil, based on probabilistic approaches, were estimated. To the best of our knowledge, ours is the first study to investigate the ecotoxicity of MEK *via* a battery of bioassays using soil fauna and flora, and to specify the soil concentrations at which MEK is

hazardous, using probabilistic ecological risk assessment approaches.

2. Materials and methods

2.1. Test materials and soils

MEK ($C_2H_5COCH_3$, CAS number 78-93-3, $\geq$ 99% purity) was obtained from Duksan Chemical (Duksan Chemical, Ansan, Gyeonggi-do Province, Korea). The test soil was organic natural LUFA (Landwirtschaftliche Untersuchungs and Forschungsanstalt) 2.2 soil obtained from Germany. The physicochemical properties of the soil are as follows: pH 6.17, organic matter (OM) 4.7%, total nitrogen (TN) 1188 mg/kg, total phosphate (TP) 265 mg/kg, cation exchange capacity (CEC) 7 Cmol + /kg, and water-holding capacity (WHC) 0.473 mL/g. MEK was diluted in distilled water and spiked in the test soils before the test. Because of the volatility of MEK (vapor pressure 78 mmHg at 20 °C) [22], ventilation was applied in each assay.

2.2. Soil algae assays

Two species belonging to the class Chlorophyceae, *Chlamydomonas reinhardtii* and *Chlorococcum infusionum*, were used in the soil algae assays, which were obtained from the University of Texas (Austin) and the University of Göttingen (Germany). In the case of *C. reinhardtii*, 2.5 g of test soil was added to a glass test tube and MEK solutions were injected at concentrations of 0, 112, 224, 1120, 2240, 4480, and 6720 mg MEK/kg dry soil (water content was 65% of the WHC). The initial algal cell density was 0.625×10^6 cells/2.5 g soil and the test conditions were 24 °C, 100 rpm, 16:8-h light:dark cycle, 4400 − 8900 lx, and a closed system. The same test conditions were used for *C. infusionum*, except for the test concentrations (0, 22, 56, 112, 168, 224, and 1120 mg MEK/kg soil). After 6 days of exposure, chlorophyll-a contents were measured to reflect algal growth. Chlorophyll-a contents were extracted with 5 mL of ethanol by vortexing for 3 s, followed by incubation at 24 °C in the dark for 3 h, and then measured using a fluorescence microplate reader (Gemini, Molecular Devices, USA; excitation 420 nm and emission 671 nm). Three replicates were conducted for each concentration. The 50% effective concentration (EC_{50}) and 10% effective concentration (EC_{10}) were calculated as toxic values of acute and chronic effects, respectively [23].

2.3. Nematode assays

A species from the class Secernentea, *Caenorhabditis elegans*, was used as the soil nematode test species following the modified test protocol of Kim et al. [24]. Dry soil (0.3 g) was placed in a well of a 24-well plate and 0.2 mL of MEK solution (0, 250, 500, 1000, 1500, and 2000 mg MEK/kg dry soil) diluted with K-medium (0.032 M KCl and 0.051 M NaCl) was added. Subsequently, 10 synchronous (aged 9–12 days) nematodes were added to the contaminated soil in each well. After 24 h, the contaminated soil from each well, including nematodes, was placed on nematode growth medium (NGM) agar plates (NaCl, 3 g/L; peptone, 2.5 g/L; agar, 17 g/L; 1 M potassium phosphate, 25 mL; 1 M $CaCl_2 \cdot 2H_2O$, 1 mL; 1 M $MgSO_4 \cdot 7H_2O$, 1 mL; cholesterol, 1 mL). After 3 h, the number of offspring was determined. Tests were conducted at 20 °C in a dark and contained environment. Because the exposure time