공학석사학위논문

Ecotoxicological Assessment of Explosives Using Soil Microbial Activity and Determination of Ecologically Permissible Soil Concentrations

토양미생물의 활성을 이용한 화약류의 토양 생태독성 및 생태학적 허용농도 결정에 관한 연구

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Abstract

Ecologically Permissible Concentrations for Protection of Soil and Water Ecosystem and Their Ecotoxicity on Soil Microbes

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Soil contamination with explosives at firing ranges are recently found to be influential to the surrounding ecosystem. At highly active firing ranges, it is more reasonable to manage the toxic effect of explosives on surrounding ecosystem than the direct remediation of contaminants on site. This study was performed to determine the effects of explosives-contaminated soil and water and to suggest ecologically permissible concentrations of explosives. Among explosives, 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) were selected as target pollutants. Moreover, microbial activity experiments were executed for more qualified derivation of permissible concentrations, since few microbial toxicity data of TNT and RDX were available.

Toxicity data of various species were available, however, the effects of TNT and RDX on microbes were not studied widely, only few soil toxicity data of TNT and RDX on microbes were available. Therefore, the study of toxic effects of TNT and RDX on microbes

is required. Soil microbial activity such as potential nitrification activity, dehydragenase activity, phosphatase activity, fluorescein diacetate activity, β-glucosidase activity, arylsulfatase activity and rhodanese activity were measured using seven different soil types. NOECs were derived to verify the toxicity of TNT and RDX on soil microbes by calculating the geometric means of NOECs using different soil types each from different enzymatic assay methods. For TNT, NOEC values varied from 45.31 (fluorescein diacetate activity) to 55.15 (dehydrogenase activity) mg/kg and for RDX, NOEC values varied from 285.9 (phosphatase activity) to 308.9 (dehydrogenase activity) mg/kg.

The derivation and suggestion of permissible concentrations of TNT and RDX are required, since there is no standards of TNT and RDX in Korea. The toxicity values of various test organisms from literatures are chosen in order to derive ecological permissible concentrations. The permissible concentrations were derived using guidelines such as 'A Protocol for the Derivation of Environmental and Human Health Soil Quality Guidelines (CCME)' and 'Guidance for the derivation of environmental risk limits within the framework of 'International and national environmental quality standards for substances in the Netherlands (RIVM)'. Ecologically permissible concentrations of TNT and RDX in soil are suggested as TNT-7.7 (UF=5) and 17.3 (UF=1) mg/kg, RDX-18.3 (UC=5) and 41 (UF=1) mg/kg for Dutch RIVM approach and TNT-5.6 (UF=5) and 28.1 (UF=1) mg/kg, RDX-15 (UF=5) and 75 (UF=1) mg/kg for Canadian CCME approach. Each concentrations were derived through selected procedure chosen by quality of each toxicity data sets. The permissible concentrations of TNT varies slightly less than RDX between CCME and RIVM approach. The toxicity data of RDX used

for CCME approach might determined to be less qualified because EC50 values were used due to lack of EC20 values. When using this data set, RIVM approach can be determined to be more precise.

For determination of environmental quality standard of each contaminants, not only toxicity but also background concentration, capability and applicability of remediation and management technology and other social/economical conditions should be considered, however ecologically permissible concentrations derived in this study can be used as screening level.

HC_5 values which are derived in this study were set to PNEC (Predicted No Effect Concentration), and the measured TNT and RDX concentrations in active firing range soil were set to PEC (Predicted Exposure Concentration). HQ (Hazardous Quotient) was calculated to determine the ecotoxicological risk. PEC were the representative concentration of TNT-40.59 mg/kg (95% Gamma UCL) and RDX-52.97 mg/kg (95% chebyshev UCL) using incremental sampling. Ecologically permissible firing range soil for protecting aquatic species of TNT-807 mg/kg and RDX-56 mg/kg were used for PNEC. The calculated HQ value of TNT was 0.05, and for RDX, it was 0.95 with representative concentrations indicating no ecotoxicological risk are found in this contamination site, however the derived HQ value of RDX was 0.95 which is close to one. It is determined that further investigation on RDX in active firing range is needed.

Keywords : TNT, RDX, ecologically permissible concentration, toxicity test on soil microbial activity, screening level ecological risk assessment

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1. Introduction

1.1 Background

Ecotoxicology is a study focuses on the effect of toxic chemicals on biological organisms which covers both ecology and toxicology. For assessing ecotoxicity of target contaminants, ecotoxicity testings such as acute or chronic tests on terrestrial and aquatic species are used. For terrestrial species, plant, avian, mammalian, invertebrate and soil microbe are representative ecological receptors, and fish, crustacean, annelid and algae are representative ecological receptors for aquatic species. Ecotoxicity of toxic compounds should be verified in order to set environmental quality standard considering ecological receptors. Derivation process of environmental soil quality standard can be described with several procedures. Enough toxicity information is needed to derive species sensitivity distributions, furthermore hazard concentrations for protecting ecological receptors are chosen from species sensitivity distribution to be used for setting soil quality standard.

Soil contamination with explosives at firing ranges could be influential to the surrounding ecosystem. Concerns about firing range contaminated soil have risen, however, it is difficult to control explosives since there is no standards or laws to manage or remediate explosives. In addition, as the interest on ecosystem getting higher, ecological risk assessment became more important. In case of soil media, some countries such as USA, Canada, Netherlands and Germany consider both risk assessment based on human health effect and ecological risk assessment based on terrestrial species. Risk assessment in active firing ranges has been performed in Korea, but

most results showed there's no significant risk on active firing ranges since most active firing ranges are unfrequented by human, however, it is possible for wildlife to approach near active firing range, therefore, terrestrial receptors should be considered and ecological risk assessment should be performed.

The derivation and suggestion of permissible concentrations of TNT and RDX are required, since there is no standards of TNT and RDX in Korea. In order to perform ecotoxicological risk assessment the toxicity information is needed. Toxicity data of various species are available, however, the effects of TNT and RDX on microbes are not studied widely, only few soil toxicity data of TNT and RDX on microbes are available. Therefore, the study of toxic effects of TNT and RDX on microbes is required. Soil microbial activity such as potential nitrification activity, dehydrogenase activity, phosphatase activity, fluorescein diacetate hydrolytic activity, β-glucosidase activity, arylsulfatase activity and rhodanese activity are major methods for assessing ecotoxicity on soil microbes and deriving toxicity endpoints.

1.2 Objectives

The principal objective of this study was to suggest ecologically permissible concentrations based on species sensitivity distribution. The following detailed objectives were established in this study.

(1) To derive ecologically permissible soil and water concentrations of TNT and RDX

(2) To assess toxicity effects of TNT and RDX on soil microbes and to suggest toxicity endpoints of TNT and RDX on soil microbes by soil microbial activity tests

(3) To derive ecologically permissible soil concentration of TNT and RDX including soil microbial activity test results

(4) To suggest ecologically permissible firing range soil concentrations of TNT and RDX by predicted efflux equation at Darakdae active firing range and to assess ecological risk at Darakdae active firing range based on ecologically permissible firing range soil concentration

1.3 Dissertation Structure

This study collected toxicity data representing toxic effects of TNT and RDX on various ecological receptors, and derived ecologically permissible concentration based on species sensitivity distribution. Additionally, toxic effects of TNT and RDX on soil microbes were assessed by soil microbial activity tests. The following detailed structure and contents are established in this study.

(1) Derivation of ecologically permissible concentration
Derive ecologically permissible soil and water concentrations using species sensitivity distribution with selected toxicity data and assess the ecological toxicity effect

(2) Assessment of TNT and RDX toxicity on soil microbes
Validate the toxicity of TNT and RDX on soil microbes through seven different soil microbial activity tests using seven different soil types

(3) Derivation of ecologically permissible soil concentration including soil microbial activity test results
Derive ecologically permissible soil concentration including NOECs of TNT and RDX on soil microbes from this study

(4) Site-specific application at Darakdae active firing range
Derive ecologically permissible firing range soil concentration using predicted efflux equation at Darakdae active firing range, and assess ecotoxicological risk on site

2. Literature Review

2.1 Ecotoxicity Tests

Ecotoxicity tests are experiments for toxicity determination on ecological organisms. Ecotoxicity tests can be divided to field test and laboratory test. Laboratory tests might overestimate the toxicity, however, it is difficult to conduct field test and to find enough field toxicity data.

2.1.1 Ecotoxicity tests on terrestrial species

To assess the toxicity of contaminants on terrestrial species, toxicity tests on soil invertebrates, plants and wildlife are performed. Toxicity test must be conducted according to the international Organizations for Standardization (ISO) and the Organization for Economic Co-operation and Development (OECD). Ecotoxicity tests are conducted using soil invertebrates, plants, soil microbes and terrestrial-dwelling wildlife in order to verify the toxicity of target contaminant in soil. Ecotoxicity tests are usually carried out with properly selected species and experimental conditions according to internationally accepted soil testing protocols such as ISO, OECD and ASTM.

When performing terrestrial toxicity tests, *Hordeum vulgare* is usually selected for representative receptor since *H. vulgare* is most widely selected species of toxicity tests (ISO 15799. A.1.2.1 method, 2003). Germination rate, fresh/dry weight, root length are usually chosen to measure for assessing toxicity of target contaminants.

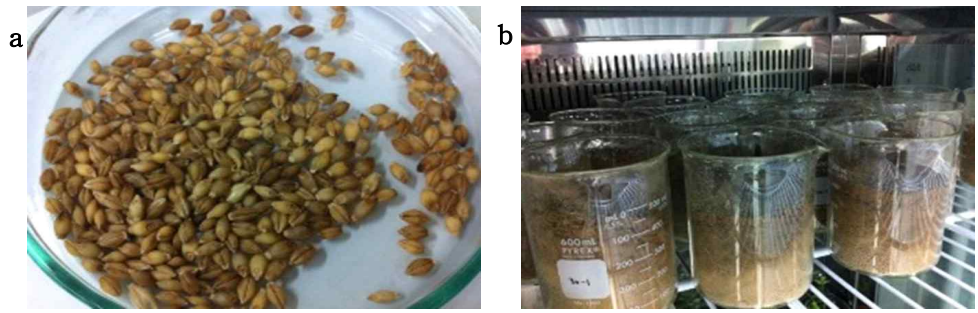


Figure 2.1. (a) *Hordeum vulgare* and (b) view of test procedure

Toxicity values such as no observed effect concentration (NOEC), low observed effect concentration (LOEC) and effect concentration (ECx) can be calculated by performing toxicity tests and deriving dose response curve (Figure 2.2).

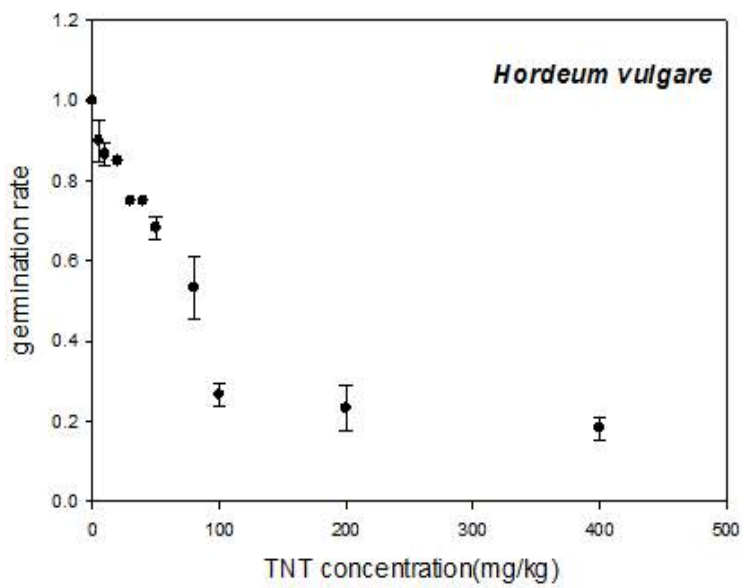


Figure 2.2. Dose-response curve of germination test

2.1.2 Ecotoxicity tests on aquatic species

The ISO and the OECD have developed standards for ecotoxicological tests on aquatic organisms. Fish, crustaceans, plant and algae are most commonly used species groups to assess aquatic toxicity of target contaminant. In case of plants in aquatic compartments, higher plants have not been commonly used for ecotoxicological tests since low organisms such as duckweed (*Lemnaceae*) possess better physiological properties like small size, high multiplication rates and vegetative propagation.

When performing aquatic toxicity tests, *Lemna minor* is usually selected for species of toxicity tests (ISO 15799. A.1.2.1 method, 2003). Germination rate, fresh/dry weight, root length are usually chosen to measure for assessing toxicity of target contaminants.

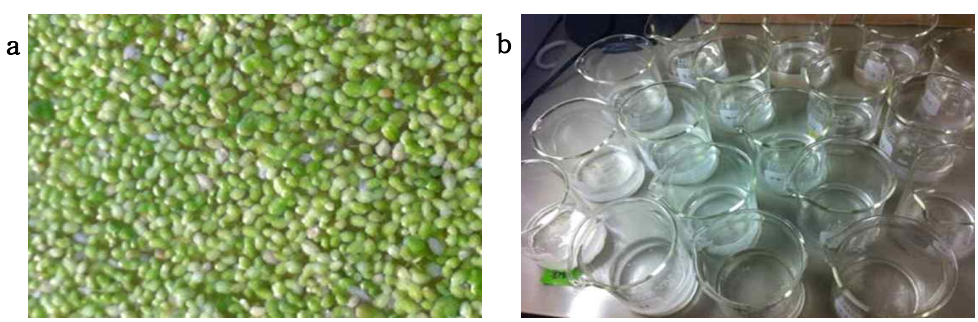


Figure 2.3. (a) *Lemna minor* and (b) view of test procedure

Toxicity values such as no observed effect concentration (NOEC), low observed effect concentration (LOEC) and effect concentration (ECx) can be calculated by performing toxicity tests and deriving dose response curve (Figure 2.4).

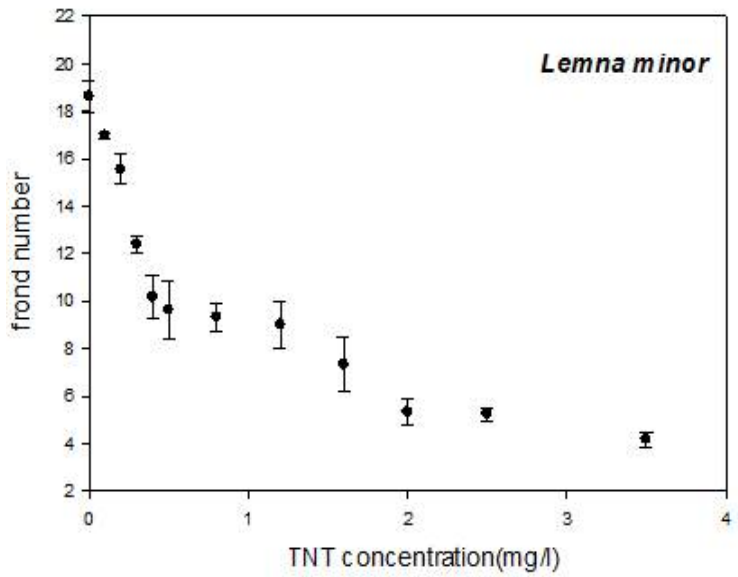


Figure 2.4. Dose-response curve of *Lemna minor* toxicity test

2.2 Ecotoxicity tests on soil microbes

Soil is a living system where all biochemical activities proceed through enzymatic processes and a biochemical processes are mediated by various living organisms such as microorganisms, plant roots and soil animals. It is important to understand the toxic effect of target contaminant on soil microbes through cognication of numbers and activities in order to verify the characteristics of soil.

Measuring the enzyme reaction activity of soil microbes can verify the toxic effect of target contaminants accurately compared to analyze soil microbial population. The toxicity test method of luminescent bacteria is studied and used widely (Drzyzga. 1995), however soil microbial activity tests are more appropriate and proper for assessing toxicity on soil microbes.

Enzyme activity differs with various conditions of temperature, pH, ionic strength and the existence of inhibitors or activators. Soil is consists of living cells available of biochemical reactions. Enzyme activity can be effected by the fraction of organic and mineral in soil.

2.2.1 Mechanism of Enzyme Action

Most chemical reactions in human body are enzyme actions, which are catalyzed by enzymes. Enzymes have specificity and catalytic efficiency, which makes a big difference from simple catalysts. With proper temperature and pH condition, enzyme actions can occur with significantly high efficiency. Enzyme action was proposed by Michaelis and Menten (1913) and can be expressed simply by the following equation

$$S + E \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

where S is the substrate, E is the enzyme, ES is the intermediate enzyme-substrate complex, P is the product of the reaction and k_1, k_2 and k_3 are the respective reaction velocity constants or rate constants of the three processes. The enzyme reaction can generally be observed by measuring the rate of chemical reaction catalyzed by the enzyme.

2.2.2 The Activity of Soil Microbes

Soil microbial activity tests were performed to assess the toxicity of TNT and RDX on soil microbes which are major component of soil. Since soil microbes are at the bottom of trophic levels in soil it is important to verify the impact on soil microbes to assess terrestrial ecotoxicity of TNT and RDX. Soil microbial activity tests were executed using seven different soil enzyme activity test procedures which are potential nitrification activity, dehydrogenase activity, phosphatase activity, fluorescein diacetate activity, β-glucosidase activity, arylsulfatase activity and rhodanese activity. Firing range soil, field soil, paddy soil, landfill soil and sand, clay, peat soil were used as different soil types for measuring the activity of soil microbes.

2.2.2.1 Potential Nitrification Activity

Most nitrates (NO_3^-) and nitrites (NO_2^-) are actually produced through the nitrogen cycle, and it consists of two parts: nitrification and denitrification. Denitrification is a specific metabolic procedure done by a limited number of bacterial genera using nitrate or nitrite as a substrate. The overall denitrification process is shown in the following equation.

$$\underset{(+5)}{NO_3^-} \rightarrow \underset{(+3)}{NO_2^-} \rightarrow [Enz\,NO] \rightarrow \underset{(+1)}{N_2O} \rightarrow \underset{(0)}{N_2}$$

Denitrification can be referred as ‘dissimilatory nitrate reduction’, where nitrate replaces oxygen as the terminal electron acceptor. Measuring the activity of nitrifying organisms participating in the

reaction of forming nitrate by oxidizing nitrite is the main principle of this potential nitrification activity test method. Denitrification is assumed to have the largest special and temporal variability of nitrogen cycle processes since denitrification is predominantly confined to active sites in aerobic soils.

2.2.2.2 Dehydrogenase Activity

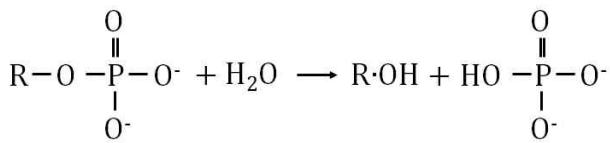
Dehydrogenase activity has executed most frequently since biological oxidation of organic compound is normally a dehydrogenation process and dehydrogenase enzyme systems are known to fulfill a significant role in the oxidation of soil organic matter. An organic compound as hydrogen donor and various hydrogen acceptor are participated in the dehydrogenase reaction.

Skujins (1973) showed that dehydrogenase activity was highly correlated with CO_2 release, proteolytic activity and nitrification potential, however, correlation with microbial numbers was not found. The dehydrogenase activity depends on the total metabolic activity of soil microbes, its values in different soils containing different populations do not always reflect the total numbers of countable microbes cultured in particular medium.

This method can distinguish the existence of alive cell by the respiration level. Determine respiratory ability and growth ability by measuring the activity of dehydrogenase attending in reaction. When dehydrogenase and colorless tetrazolium solution are together, hydrogen ion from dehydrogenase combine with tetrazolium to form red color formazan.

2.2.2.3 Phosphatase Activity

Enzymes participate in this reaction are related to acid phosphatase in soils, and known to hydrolyze a variety of phosphomonoesters. The generalized equation of the reaction catalyzed by acid phosphatase is shown in following equation.



The activity of phosphatase has been found in various plants, animals, and microorganisms and best known for its ability to degrade nucleic acid and given the demonstrated ability of soils to decompose added nucleic acids and the natural occurrence of P compounds. This method uses the hydrolysis property of phosphatase to assess the activity of indigenous microbes in soil, involving colorimetric estimation of the *p*-nitrophenol released when soil is incubated with buffered sodium *p*-nitrophenyl phosphate solution and toluene. Orthophosphate are formed by accumulated phosphate in soil by hydrolysis-able phosphatases. The colorimetric procedure used for estimation of *p*-nitrophenol is based on the fact that alkaline solutions of phenol have a yellow color when acid solutions of *p*-nitrophenol and *p*-nitrophenyl phosphate are colorless.

2.2.2.4 Fluorescein Diacetate hydrolytic Activity

Fluorescein diacetate hydrolysis method is widely used for measuring microbial enzyme activities since most enzymes can hydrolyze fluorescein diacetate (3-6 diacetyl fluorescein) and produce fluorescein diacetate. Fluorescein diacetate hydrolytic activity can be used to determine total microbial activity, this method is a good generic measure of organic matter turnover in nature because more than 90% of the energy flow passes through microbial decomposers. Moreover all fungi, most bacteria, protozoa and algae can show fluorescein diacetate hydrolytic activity (Schnurer et al., 1982).

Living organisms can convert the non-fluorescent fluorescein diacetate in to the green fluorescent compound which is fluorescein. Red glow represents a sign of cell death while a bright yellow glow is produced when enzymatic activity is great.

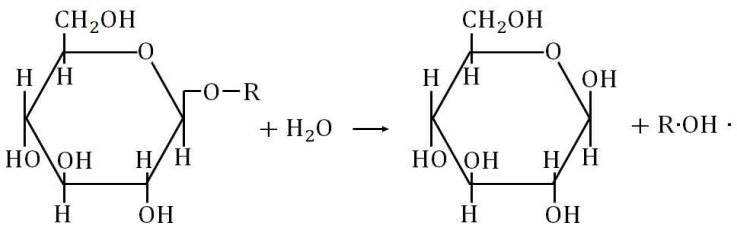
2.2.2.5 β-glucosidase Activity

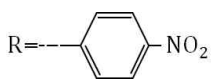
Glucosidases are detected in soils and widely distributed in nature in fungi, yeast, and plants, in addition the enzymes using glycosyl compounds such as glycoside hydrolases have been studied in soils. Glycosidases like *glycosidases or glycoside hydrolases* have usually been named according to the bond type they hydrolyze, β-glucosidase catalyzes the hydrolysis of β-D-glucopyranosides and involved in hydrolysis of cellobiose. The general reaction is shown below.

$$Glycosides + H_2O \rightarrow Sugar + Aglycons$$

Glycoside can be defined generally as a mixed acetal from the exchange of an alkyl or aryl group for the hydrogen atom of the hemiacetal hydroxyl group. The aglycon is also known as genin and is the noncarbohydrate portion of glycoside.

β-glucosidases are widely distributed in nature, in fungi (Jermyn, 1958), yeast (Barnett et al., 1056) and plants (Veibel, 1950). In addition, *β*-glucosidase is more dominant than other enzymes such as α-glucosidase, α-galactosidases and β-galactosidases and the products produced after hydrolysis reaction are important energy sources for soil microbes. The principle of *β*-glucosidase activity is determination of *p*-nitrophenol released by *β*-glucosidase based on colorimetric assay. The representing reaction is shown below,



where, p-nitrophenyl-β-D-glucoside is substrate and 
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2.2.2.6 Arylsulfatase Activity

Sulfatases can be classified by the type of organic sulfate esters hydrolyzed; arylsulfatases, alkylsulfatases, steroid sulfatases, glucosulfatases, chondrosulfatases and myrosulfatases. Among all, arylsulfatase which arylsulfate sulfohydrolase is most commonly used for microbial activity tests since this enzyme was first detected in nature. Arylsulfatase is the enzyme that catalyzes the hydrolyses of an arylsulfate anion by the fission of O-S bond, the reaction is shown below.

$$R \cdot OSO_3^- + H_2O = R \cdot OH + H^+ + SO_4^{2-}$$

This reaction is irreversible and partially responsible for S cycling in soils. The suggested role of this enzyme is to mineralize the ester sulfate in soil to make it usable for plants to grow and be participated in S cycle.

2.2.2.7 Rhodanese Activity

Rhodanese which is thiosulfate-cyanide sulfuransferase, can catalyze the formation of thiocyanide (SCN^-) from the thiosulfate ($S_2O_3^{2-}$) and cyanide (CN^-).

$$S_2O_3^{2-} + CN^- \rightarrow SCN^- + SO_3^{2-}$$

Rhodanese was first discovered in animal tissues and detected widely in nature. $S_2O_3^{2-}$ is an intermediate S compound produced during oxidation of elemental S in soils (Nor and Tabatabai, 1977). Tabatabai and Singh (1979) has found kinetic parameters of rhodanese reaction in soil showed the Km values of $S_2O_3^{2-}$ and CN^- to be similar to those for the same enzyme isolated from other biological systems. This method is based on colorimetric determination of the SCN^- produced when soil is incubated with buffered $Na_2S_2O_3$ and KCN solutions and toluene, specifically the reaction of SCN^- with Fe^{3+} in acidic medium to form an Fe-SCN colored complex which can be measured at 460 nm.

2.3 Ecotoxicological Risk Assessment

Ecotoxicological risk assessment is to discern risk of target contaminant on ecological receptors and the ecotoxicological risk is estimated based on the ecotoxicity data from toxicity tests. In ecotoxicological risk assessment bioaccumulation and food web are not considered but only toxic effects are considered.

Data extrapolation types should be selected considering the size and quality of collected toxicity data since it is important to use flexible approaches to deal uncertainties in laboratory tests. There are two different data extrapolation method which are probabilistic approach and deterministic approach.

2.3.1 Probabilistic approach

Probabilistic approach is preferred in order to reflect the richness of species diversity and critical ecosystem functions in the real environment. Probabilistic approach can be used when there are enough qualified toxicity data. There are two major types of distribution-based extrapolation methods, the first method is a ranked frequency distribution approach and the second is a statistical approach, which is a species sensitivity distribution. Species sensitivity distribution was developed as an ecotoxicological tool for deriving environmental standards and to quantify ecological risk of target contaminants on ecological receptors by ecological risk assessment.

2.3.1.1 Species sensitivity distribution

Species Sensitivity Distribution (SSD) is a statistical distribution representing the toxicity variation of target contaminants on a set of species. It is evident that the number of data is highly important for deriving species sensitivity distribution. Species sensitivity distribution is estimated from a set of various toxicity data and visualized as a cumulative distribution function, since it is difficult to know the true distribution of toxicity endpoints. A cumulative distribution function is the integral of a probability density function. Cumulative distribution function consists of sensitivity data from acute or chronic ecotoxicological tests.

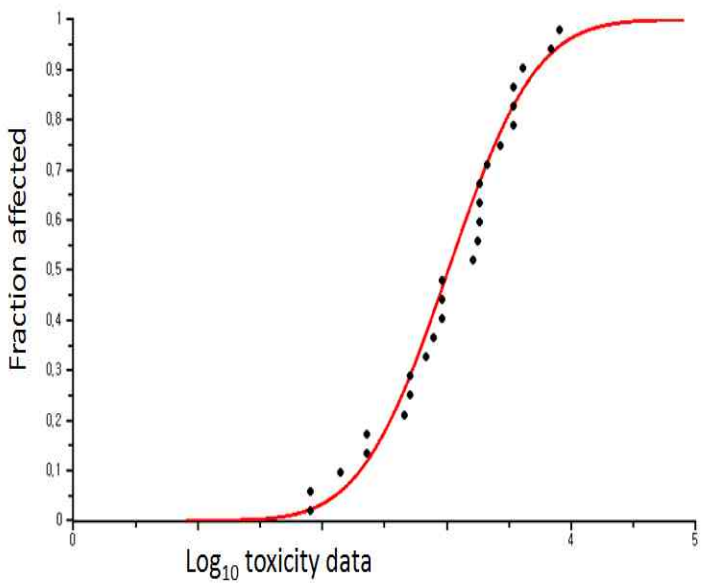


Figure 2.5. Species sensitivity distribution

X-axis in the graph (Figure 2.5) indicates the target contaminant concentration and Y-axis shows the percentage of effected species at a certain concentration. Species sensitivity distribution can be interpreted in both 'forward' and 'inverse' way.

Environmental quality criteria can be derived by using the inverse use, a cutoff percentage p is chosen (protecting $1-p$ percent of species), and the concentration HC_p is calculated as a result. Although the value of p is a policy decision and can be flexible according to sites and economic level, however, HC_5 (95% protection criterion) has chosen universally.

For the forward use, ecological risk assessment can be conducted by derivation of potentially affected fraction at a target concentration or quality standard. If effects on more than 5% of species are unacceptable (HC_5), any concentration higher than HC_5 can be considered to pose a significant risk.

2.3.2 Deterministic approach

Deterministic approach is used to make maximum use of information since probabilistic approach cannot be used if there is no or little toxicity data available. It is evident that deterministic approach has higher uncertainty, therefore usually more conservative results are derived to lessen uncertainty.

2.3.2.1 Assessment factor method

Assessment factor method is suggested by several agencies such as United States Environmental Protection Agency (US EPA), Netherlands National Institute for Public Health and the Environment (Dutch RIVM), Canadian Council of Ministers of the Environment (CCME) and European Council Technical Guidance Document (EC TGD). Assessment Factors (AF) are applied to the lowest determined effect concentration mostly from laboratory toxicity data. The assessment factor represents the uncertainty of extrapolating laboratory toxicity test data, and the factors are orders of magnitude. Assessment factors are applied depending on the quantity and quality of data collected. Table 2.1 shows assessment factors suggested by Dutch RIVM for terrestrial species.

Table 2.1. Different assessment factors decided by available information

Available information	Assessment factor
L(E)C50 short-term test(s) (e.g. plants, earthworms, or microorganisms)	1000
NOEC for one long-term toxicity test (e.g. plants)	100
NOEC for additional long-term toxicity tests of two trophic levels	50
NOEC for additional long-term toxicity tests for three species of three trophic levels	10
Field data/data of model ecosystems	case-by-case

Assessment factor method can be applied to small size data sets, easy to use, and transparent, however, the magnitude of assessment factor usually does not consider ecotoxicological properties). The assessment factors suggested for the terrestrial organisms are not based on comprehensive experience but are mostly derived directly from the factors used for aquatic compartment.

2.3.2.2 Equilibrium partitioning method

The equilibrium partitioning method uses the aquatic toxicity data and the sediment/water partitioning coefficient with some assumptions, which are sediment-living organisms and aquatic organisms are equally sensitive to target contaminant, concentration of sediment, interstitial water and benthic organisms are thermodynamic equilibrium (all concentrations can be predicted using partitioning coefficients) and sediment/water partitioning coefficients can be derived by generic partitioning method from separately measurable sediment characteristics and chemical properties. This method was first developed to assess toxic effects to species living in the sediment compartment.

Equilibrium partitioning method converts aquatic data to terrestrial data using soil/water partitioning coefficient, this method can be useful since terrestrial toxicity data is often very limited compared to aquatic toxicity data. Not only toxicity data but Predicted No Observed Concentration (PNEC) can also be calculated by equilibrium partitioning method, however, high uncertainty always exists when using this method.

$$PNEC_{soil} = \frac{K_{soil-water} * PNEC_{water} * 1000}{P_{soil}}$$

$PNEC_{water}$	predicted no effect concentration in water	mg/l
P_{soil}	bulk density of wet soil	kg/m^3
$K_{soil-water}$	partition coefficient soil/water	m^3/m^3
$PNEC_{soil}$	predicted no effect concentration in soil	mg/kg

Equilibrium partitioning method was evaluated and validated by comparing aquatic toxicity data and terrestrial toxicity data of 12 organic substances and 8 metals by Dutch RIVM and they concluded that equilibrium partitioning method can overestimate or underestimate results. According to Dutch RIVM, equilibrium partitioning method is not a scientifically valid to derive environmental quality standards.

2.4 Environmental Quality Standards

2.4.1 Setting Soil Quality Standard

Generally, methods of deriving soil quality standards of numerous international approaches have similarities in overall procedure and differences in screening and selecting toxicity data. For fitting and extrapolating toxicity endpoints to derive soil quality standards, terrestrial species are classified to plant/invertebrate, microbe and wildlife regarding soil-dependant organisms might have different soil conditions for each residue.

2.4.1.1 Canadian CCME approach

Canadian CCME approach classifies contaminated sites by land use, therefore each different soil quality guidelines are suggested according to land use. There are different land use types which are agricultural/residential, parkland and industrial/commercial. Soil quality guidelines of CCME are based on effective toxicity data using mortality, reproduction and growth endpoints for soil-dwelling species such as microbes, plants, invertebrates. For agricultural land use, soil and food ingestion pathway (food chain) is considered for consumers like wildlife and livestock. The CCME soil quality guidelines are proposed based on toxicological information and other scientific data in calculation and derivation steps, and non-scientific factors such as technological, political and socio-economic factors are considered by site managers as a part of risk management process. Soil quality guidelines are preferably set to 75^{th} percentile of a frequency distribution of selected toxicity data.

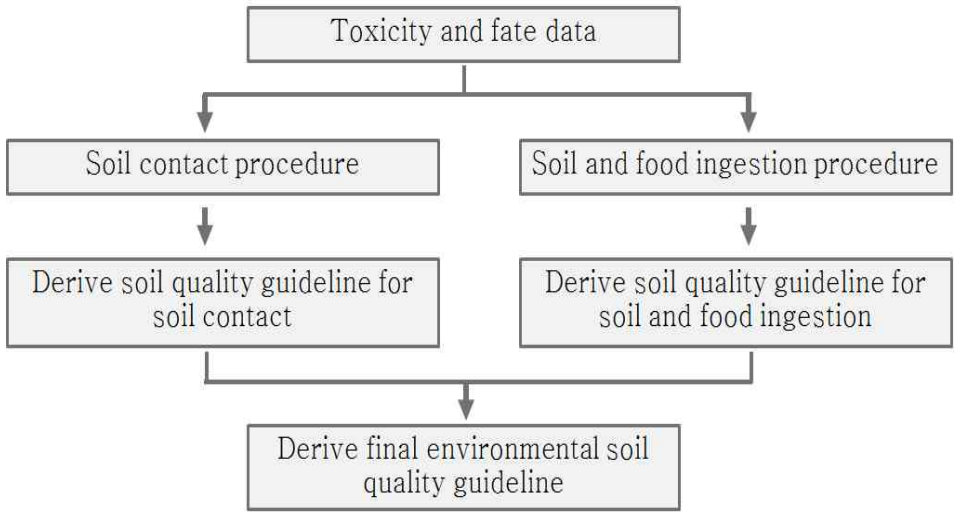


Figure 2.6. The overall procedure for derivation of soil quality guideline using Canadian CCME approach

2.4.1.2 Dutch RIVM approach

Netherlands derive soil protection values for protecting 95% of theoretical species in ecosystem considering ecological risk. The overall procedure for deriving environmental quality standards for soil protection is shown in Figure 2.7. Dutch RIVM suggests intervention value and target value for soil protection. Intervention values are set at 50^{th} percentile protection level for terrestrial species, and if the contamination concentration is above the intervention level, a site-specific risk assessment for establishing target value is needed. The target value is environmental quality standards and set to 95^{th} percentile protection level.

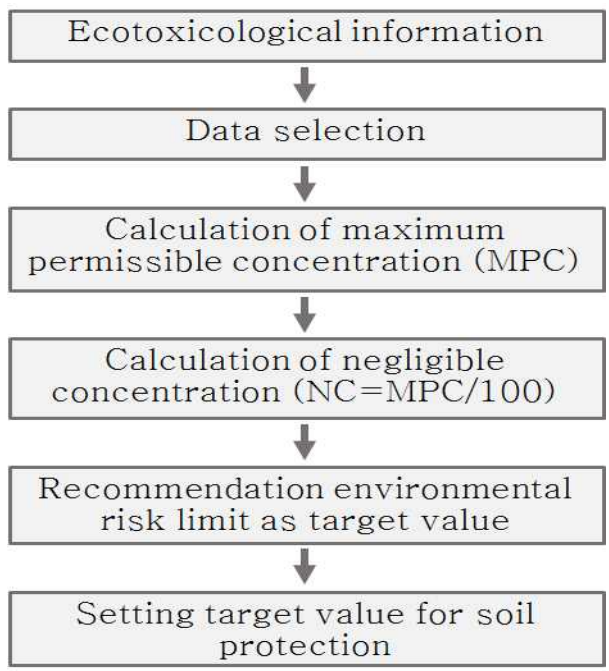
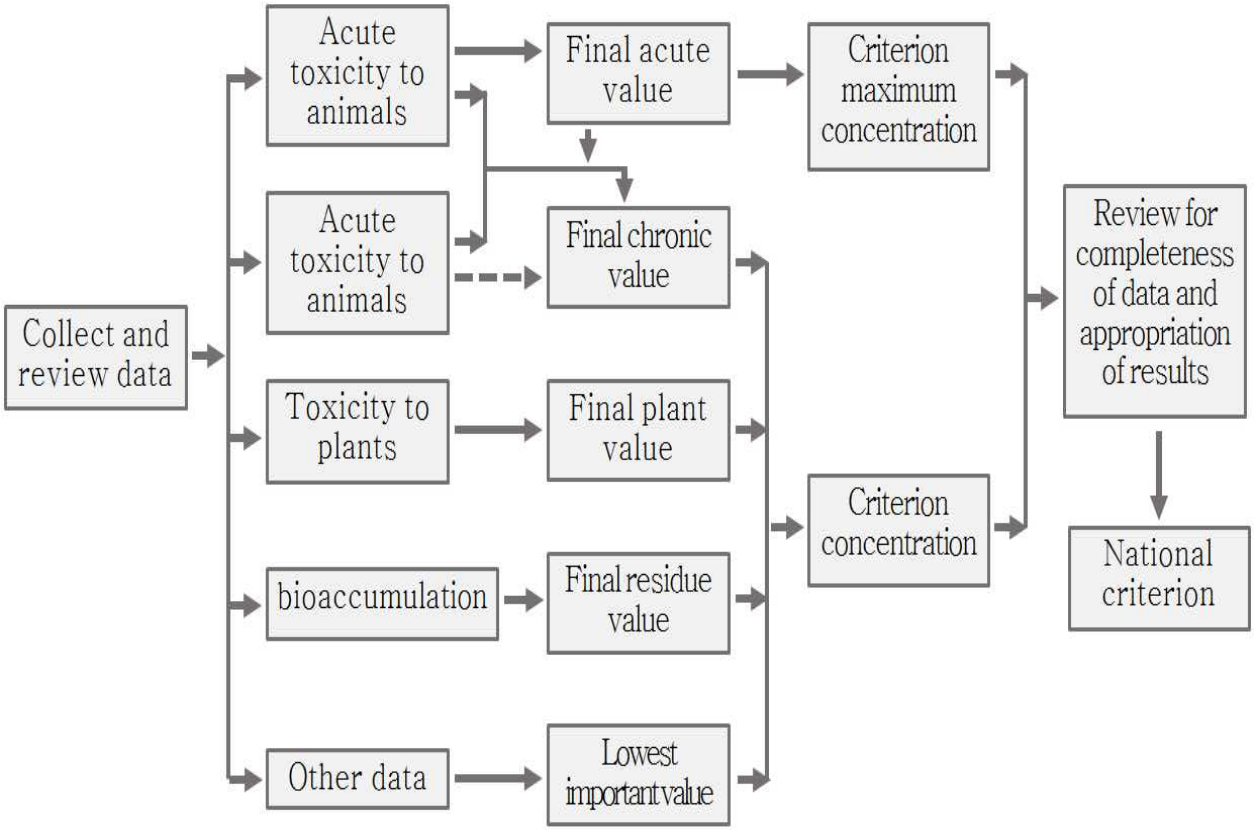


Figure 2.7. The overall procedure for derivation of environmental quality standards using Dutch RIVM approach

2.4.2 Setting Water Quality Standard

Derivation of various international water quality standards for protecting aquatic species uses numerous aquatic toxicity information from many areas of aquatic toxicology and is a complex process including data screening step. Aquatic toxicity data especially on aquatic plants are examined to determine if the contaminant concentration cause unacceptable effects to plants but not to animals. Other toxicity data are derived for adverse effects which might be biologically important. If enough acceptable toxicity informations are available, water quality criteria can be derived for fresh water or salt water or both to protect aquatic species and can be used for both short term and long term toxicity effects.

Figure 2 .8 . Overall derivation procedure for environmental water quality criteria



3. Materials and Methods

3.1 Experimental Methods

3.1.1 Materials

3.1.1.1 Test soil types

Seven different types of soil were used in this study. Seven types of soil were used, which are four different land uses such as firing range soil, field soil, paddy soil and landfill soil in addition, sand, clay, peat soil for different soil textures. Firing range soil was collected from an active firing range located in Korea, field soil was sampled from Gwanak Mountain in Korea, paddy soil was gathered from a rice paddy located in Korea and landfill soil was sampled from a landfill in Korea. Sampled soils were air-dried, passed through a 2-m sieve (#10 sieve), mixed homogeneously and the original water contents were maintained. The physicochemical properties of soils are listed in Table 3.1. The analysis of physicochemical properties of peat soil was not available due to lack of sample amount and analysis of couple more factors was not available due to other properties of soil.

Table 3.1. Physical properties of selected soil

	firing range soil	field soil	paddy soil	landfill soil	sand	clay
soil texture	sandy loam	loamy sand	silt	silt	sand	clay
pH	5.5	5.67	4.3	7.67	7.53	10.4
organic content	0.02	0.016	0.04	0.07	0.08	0.18
bulk density	2.59	2.57	1.33	2.67	2.61	-
water content (%)	35	47	40	15	53	15
EC	0.13	0.96	7.41	83.8	0.087	-
TOC	1.18	0.96	0.58	0.055	0.04	0.103

3.1.1.2 Chemicals and reagents

This study selected 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine(RDX) among explosives for a better application since 1,3,5,7-tetranitro-1,3,5,7-tetrazocane (HMX) is not used in Korea. TNT and RDX were selected as target contaminants for the representatives of nitroaromatic and nitramine explosives, respectively. Although both explosives and heavy metals found on firing ranges, this study focuses on explosives because many researches already proceeded for heavy metals and there are standards of most heavy metals. TNT and RDX were obtained from Hanwha corporation with the permission of the Korea Defense Acquisition Program Administration. The chemical structures and physical properties of TNT and RDX are shown in Table 3.1. The organic chemicals were dissolved in acetonitrile and kept in refrigerator. Massive amount of TNT (60 g/L in acetonitrile) and RDX (100 g/L in acetonitrile) were kept and for further dilution, deionized water was used.

3.1.2 Soil Microbial Activity Test Methods

Soil microbial activity tests were performed to assess the ecotoxicity of TNT and RDX on soil microbes which is a major component of soil and the base of other terrestrial species. Measuring the enzyme reaction activity of soil microbes can verify the toxic effect of target contaminants accurately compared to analyze soil microbial population. Only a few assay methods have been conducted and thoroughly evaluated to be standard method although a large number of enzyme catalyzed reactions in soils have been studied. This study selected seven different microbial activity test methods which are potential nitrification activity, dehydrogenase activity, phosphatase activity, fluorescein diacetate activity, β-glucosidase activity, arylsulfatase activity and rhodanese activity.

3.1.2.1 Potential nitrification activity

Potential nitrification activity test was performed according to Torstensson (1993) and Gong P (1999) with minor modifications. 5 g of moist soil were weighed into a 50 ml teflon tube with 100 ml of test medium (4 mM $(NH_4)_2SO_4$, 15 mM $NaClO_3$, 1 mM potassium phosphate buffer, pH 7.2). The soil slurry was agitated at 25℃ and 175 rpm for 10 hour. After 2 and 10 h of incubation, 2 ml of samples were displaced with 4 M KCl in order to terminate ammonium oxidation. After 10 h incubation, swirl the tube for 10 seconds and filter the soil suspension through a SmartPor® GHP Syringe Filter (25 mm, 0.45 μm). Measure the yellow color intensity using OPTIZEN 2120UV UV/Vis Spectrophotometer.

3.1.2.2 Dehydrogenase activity

2-(p-iodophynyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium (INT) assay was used for measuring dehydrogenase activity (Gong P. 1997). Put 2.5 g of soil (<2 mm) in a 50 ml teflon tube, add 2.5 mL of 0.1% (w/v) INT solution (0.5 M Tris buffer, pH 7.6) and swirl the tube for 10 seconds to mix the contents, stopper the tube and incubate at 37℃ for 24 hours. 10 mL of tetrahydrofuran (HPLC grade) was added to extract the formed formazan (INF), then swirl the tube for 10 seconds and filter the soil suspension through a SmartPor® GHP Syringe Filter (25 mm, 0.45 μm). Measure INF at 436 nm using OPTIZEN 2120UV UV/Vis Spectrophotometer.

3.1.2.3 Phosphatase activity

Place 1 g of soil (<2 mm) in a 50 ml teflon tube, add 0.2 ml of toluene, 4 ml of MUB (dissolve 12.1 g of tris (hydroxymethyl) aminomethoane (THAM), 11.6 g of maleic acid, 14.0 g of citric acid, and 6.3 g of boric acid in 488 ml of 1 N sodium hydroxide and dilute the solution to 1 L with water, pH 6.5), 1 ml of p-nitrophenyl phosphate solution in same buffer, and swirl the tube for 10 seconds to mix the contents, stopper the tube and place it in an incubator at 37℃ for 1 hour. After 1-hr incubation, remove stopper, add 1 ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 M NaOH, swirl the tube for 10 seconds and filter the soil suspension through a SmartPor® GHP Syringe Filter (25 mm, 0.45 μm). Measure the yellow color intensity using OPTIZEN 2120UV UV/Vis Spectrophotometer.

3.1.2.4 Fluorescein diacetate hydrolytic activity

Place 1 g of soil (<2 mm) in a 50 ml teflon tube, add 50 ml of 60 mM sodium phosphate buffer (pH 7.6) and 0.5 ml of 4.9 mM FDA lipase substrate solution (20 mg FDA lipase substrate in 10 ml acetone). wirl the tube for 10 seconds to mix the contents, stopper the tube and place it in an incubator at 37℃ for 3 hour. After 3-hr incubation, remove stopper, add 2 ml of acetone, swirl the tube for 10 seconds and filter the soil suspension through a SmartPor® GHP Syringe Filter (25 mm, 0.45 μm). Measure the intensity using OPTIZEN 2120UV UV/Vis Spectrophotometer at 490 nm.

3.1.2.5 *β*-glucosidase activity

Put 1 g of soil (<2 mm) in a 50 ml teflon tube, add 0.25 ml of toluene, 4 ml of MUB (0.5 M $CaCl_2$ and standard *p*-nitrophenol solution, pH 6.0), 1 ml of PNG solution (*p*-nitrophenyl-*β*-D-glucoside solution, 0.025 M(0.377 g PNG in 50 ml MUB, pH 6.0)). Swirl the tube for 10 seconds to mix the contents, stopper the tube and place it in an incubator at 37℃ for 1 hour. After 1-hr incubation, remove stopper, add 1 ml of 0.5 M $CaCl_2$ and 4 ml of 0.1 M THAM buffer (0.1 M tris(hydroxymethyl)aminomethane buffer, pH 12), swirl the tube for 10 seconds and filter the soil suspension through a SmartPor® GHP Syringe Filter (25 mm, 0.45 μm). Measure the yellow color intensity using OPTIZEN 2120UV UV/Vis Spectrophotometer.

3.1.2.6 Arylsulfatase activity

Place 1 g soil (<2 mm) in a 50 ml teflon tube, add 0.25 ml of toluene, 4 ml of acetate buffer, and 1 ml of *p*-nitrophenyl sulfate solution, and swirl the flask for 10 seconds. Stopper the tube and place it in a 37℃ incubator for 1 hour. After 1 hour, add 1 ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 M NaOH, swirl the tube for 10 seconds and filter the soil suspension through a SmartPor® GHP Syringe Filter (25 mm, 0.45 μm). Measure the yellow color intensity using OPTIZEN 2120UV UV/Vis Spectrophotometer.

3.1.2.7 Rhodanese activity

Put 4 g soil in a 50 ml teflon tube, add 0.5 ml toluene, 8 ml of THAM buffer, 1 ml of 0.1 M $Na_2S_2O_3$ and 1 ml of 0.1 M KCN. After swirling for 10 seconds, stopper the tube and incubate for 1 hour (37℃). After 1 hour, remove the stopper and add 10 ml of $CaSO_4$-formaldehyde solution. Swirl the tube for 10 seconds and filter the soil suspension through a SmartPor® GHP Syringe Filter (25 mm, 0.45 μm). Measure the yellow color intensity using OPTIZEN 2120UV UV/Vis Spectrophotometer.

3.2 Research Methods

3.2.1 Toxicological Data Qualification Process

Toxicological data for deriving ecologically permissible concentration were chosen by toxicological data qualification process, through data collection, screening and qualification step.

3.2.1.1 Data collection

Toxicological data of TNT and RDX were collected from ECOTOX of US EPA, Validation of Environmental Military Threshold Values for Explosives in Soil of SCMTS (2008), Wildlife Toxicity Assessment for TNT of USACHPPM (2001), Wildlife Toxicity Assessment for RDX of USACHPPM (2002), A Protocol for the Derivation of Environmental and Human Health Soil Quality Guidelines of CCME (2006) and additional technical papers. Collected toxicity endpoints were consists of both acute and chronic data of terrestrial and aquatic compartments. There were plant, soil microbes, soil invertebrates and wildlife as a terrestrial species group, fish, crustacean, plant and algae as a major aquatic species group (Table 3.2).

Table 3.2. The number of collected toxicity data

Media	Contaminants	Trophic level	Values	Species
Terrestrial	TNT	Plant/Invertebrate	207	41
		Microbe	17	11
	RDX	Wildlife	21	5
		Plant/Invertebrate	75	32
		Wildlife	35	14
Aquatic	TNT	Plant	41	12
		Fish	27	6
		Crustacean	25	9
		Algae	17	4
		Annelida/Echinoderm/ Rotifer/Invertebrate	19	6
		Amphibian	2	1
	RDX	Fish	30	8
		Crustacean	25	9
		Algae	16	4
		Echinoderm/Microbe	2	1

3.2.1.2 Data screening

Collected data from references were not validated, therefore screening procedure is needed in order to guarantee the reliability. The bioassay test procedure used for deriving each toxicity information should be conducted by accepted soil toxicity testing protocols and should consider changes of experiment conditions such as temperature or pH changes. In addition, the reference much contain clear statements of exposure time, toxicological endpoint, statistical analysis, actual spiked concentration and experimental conditions such as organic, clay, water content of soil, soil type, soil pH and temperature. Analysis method of target contaminants should be acknowledgeable and comparable and experimental effect must be attributable to a target contaminant only. Table 3.3 contains the number of screened toxicity data of TNT and RDX.

Table 3.3. The number of screened toxicity data

		TNT		RDX	
		Values	Species	Values	Species
Terrestrial	Plant/Invertebrate	96	23	55	20
	Microbe	10	4	0	0
	Wildlife	21	5	35	14
Aquatic		87	28	60	19

3.2.1.3 Data qualification

In case of terrestrial toxicity informations, normalization process must be conducted to reflect the effect of different soil types. Ecotoxicological effect can be changed by organic matter content, therefore, normalization of each endpoints can be done using following equation when the organic matter content of standard soil is 3.4%.

$$NOEC \text{or} L(E)C_{x(standard)} = NOEC \text{or} L(E)C_x \cdot \frac{Fom_{soil(standard)}}{Fom_{soil}}$$

After normalization, deriving representative endpoint is needed. One representative endpoint is derived for each endpoint of each species. Most sensitive endpoint can be used as representative endpoint or the geometric mean of several toxicity data of same endpoints on same species. The number of toxicity data after qualification is shown in Table 3.4.

Table 3.4. The number of qualified toxicity data

		TNT		RDX	
		Values	Species	Values	Species
Terrestrial	Plant/Invertebrate	45	16	37	13
	Microbe	4	4	0	0
	Wildlife	5	5	16	11
Aquatic		57	40	34	23

3.2.1.4 Data extrapolation type selection

Collected data should be extrapolated to derive a significant value for using it as the ecologically permissible concentration. Since there is a paucity of terrestrial toxicity information of TNT and RDX data extrapolation process is needed in order to adjust laboratory data and to reflect functions and richness of diversity on the real ecosystem (Samantha F, 2004).

3.2.2 Species Sensitivity Distribution (SSD)

Species sensitivity distribution can be derived by extrapolating the cumulative distribution function of numerous toxicity data of each contaminants from several species. From the derived species sensitivity distribution, the effected species percentage on a certain contaminant concentration can be found as well as ecologically permissible concentration satisfying selected protection percentage.

3.2.2.1 The concept of SSD

Species sensitivity distribution is a graph where the toxic effect of various species on target contaminant statistically expressed. After toxicity data are shown in cumulative distribution function, one of several extrapolation types is selected for curve fitting. Normally log-normal distribution is selected among normal distribution, log-normal distribution and log-logistic distribution since extrapolation with higher certainty can be conducted when using log-normal distribution compared to normal distribution, moreover when compared to log-logistic distribution, log-normal distribution can be applied

flexibly compared to log-logistic distribution and mathematically simple. USEPA as well as Dutch RIVM use log-normal distribution for SSD derivation.

In this study, long-normal distribution is selected to construct species sensitivity distribution. The following equations show probability distribution function (f) and cumulative distribution function (CDF) of log-normal distribution.

$$f(x;\mu,\sigma) = \frac{1}{x\sigma\sqrt{2\pi}} e^{-(\ln x - \mu)^2/2\sigma^2}$$

$$CDF(x;\mu,\sigma) = \frac{1}{2} + \frac{1}{2} erf[\frac{\ln(x) - \mu}{\sigma\sqrt{2}}]$$

$$erf(x) = \frac{2}{\sqrt{x}} \int_0^x e^{-t^2} dt$$

where μ and σ are mean and standard deviation of the variable logarithm, respectively.

3.2.2.2 Hazardous concentration and fraction affected

HC_5 (Hazardous Concentration) can be derived from species sensitivity distribution indicating the maximum concentration for the protection of 95% species (Wheeler et al., 2002). HC_5 can be regarded as precautionary cut-off value which found to be conservative compared to quality standards. The protection percentage is flexible according to site specific conditions and economic levels, for example when the target protection receptor is endangered species the protection level must be changed by the ecotoxicological effect on target endangered species. In addition, if the contaminated site is unaccessible to wildlife the protection percentage can be lower than 95%, however 95% is selected universally in the standard derivation procedure.

3.2.3 Derivation of Ecologically Permissible Concentration

PNEC is Predicted No Effect Concentration indicating selected concentration is ecologically safe to receptors. PNEC can be determined from HC_5 divided by AF (Assessment Factor). AF can be chosen differently considering target contaminated site conditions and properties of target contaminants. Regarding the ecological toxicity of TNT and RDX, AF was set to 1 indicating PNEC to be same as HC_5 and ecologically permissible concentration in this study.

3.2.3.1 Ecologically permissible soil concentration

Toxicity data on terrestrial species were collected from references, and screened, qualified using both Canadian CCME approach and Dutch RIVM approach. After screening and qualification, verified terrestrial toxicity data were divided to three categories, plant/invertebrate, microbe, wildlife and species sensitivity distributions were derived respectively. The most conservative value among plant/invertebrate, microbe, wildlife HC_5 values was chosen as ecologically permissible soil concentration.

3.2.3.2 Ecologically permissible water concentration

Aquatic toxicity data were collected from references, and screened using both Canadian CCME approach and Dutch RIVM approach. Screened toxicity data on aquatic species were used to derive a species sensitivity distribution.

3.3 Analysis

3.3.1 Soil Microbial Activity Analysis

3.3.1.1 Ion Chromatography (IC)

Ion Chromatography (Dionex ICS-1100, Thermo, USA) was used for analyzing nitrite and nitrate concentration for potential nitrification activity using anion eluent (0.168 g sodium bicarbonate, ACS reagent, 99.7-100.3% and sodium carbonate, ACS reagent, anhydrous 0.742 g in 2 L deionized water).

3.3.1.2 UV/Vis Spectrophotometer

Most microbial activity test results were derived using UV/Vis Spectrophotometer (OPTIZEN 2120 UV) since six out of seven microbial activity tests were colorimetric assay. The analysis wavelength of each dehydrogenase activity, phosphatase activity, fluorescein diacetate activity, β-glucosidase activity, arylsulfatase activity, rhodanese activity was 436, 540, 490, 540, 540 and 460 nm, respectively.

3.3.2 Statistics and Data Interpretation

All experimental data were expressed in a dry-weight base of soil. Dunnett's test was used to derive the NOEC. Dunnett's test is conducted by computing a two-tailed t test for each experiments.

3.3.3 TNT and RDX Analysis (HPLC)

TNT and RDX was analyzed by High Performance Liquid Chromatography with separation module (Dionex Ultamate 3000), Gemini column (150 x 4.6 m, NX C18 column) and Security Guard column (Phenomenex) using the U.S. Environmental Protection Agency Method 8330 for TNT and for RDX, 18-h sonication extraction with acetonitrile was done. The operation condition of HPLC is shown in Table 3.5.

Table 3.5. The operation conditions of HPLC

Parts	Conditions		Unit
	TNT	RDX	
Column	Gemini (,150 x 4.6 mm, NX C18)		
Injection volume	9		μL
Initial column temperature	30		℃
Flow rate of carrier fluid	1.5		mL/min
Isocratic elution condition	70% methanol : 30% water		
Analysis wavelength	230	230	nm
Detection time	17.147	7.193	min
Detection limit	0.004	0.001	mg/L

4. Result and Discussion

4.1 Soil Microbial Activity

As a general consideration on soil function, accumulation of the toxic substances through food chain and effects on plant biomass production are included as adverse effects on terrestrial environment. Toxicity data of TNT and RDX for various terrestrial species such as plants, soil invertebrates and wildlife are available, however, only few soil toxicity data of TNT and RDX is available. Since soil microbes are major components of soil and occupy at the bottom of trophic levels in soil they should be considered in order to assess the toxicity effects on soil function. Generally,

4.1.1 Soil Microbial Activity Test Results

With higher TNT or RDX concentration, the microbial activity has found to be low compared to that of lower TNT or RDX concentration indicating that the indigenous microbes got affected by the toxicity of TNT or RDX. Soil microbial activity tend to decrease after a certain point. With the longer exposure time, both TNT and RDX using all seven different enzymatic methods seemed to have higher toxicity on soil microbes. Microbes participated in enzyme reactions were decided to be no longer functional or alive since most NOECs became constant after 4 weeks. Moreover 8 weeks-exposure can be regarded long enough for assessing long-term toxicity of TNT and RDX on soil microbes, therefore it is predicted that NOEC values of TNT and RDX stay constant after 8 weeks-exposure.

NOEC values of firing range soil is relatively high compared to other soil types even though firing range soil has comparably low pH, indicating firing range indigenous microbes might have tolerance on TNT and RDX.

Some physical properties of soil such as pH, soil texture and organic content seemed to have affected the toxicity revelation of TNT and RDX on soil microbes. Therefore, some statistical analysis were conducted to verify the effects of factors on soil microbial activity. Through factor analysis, the relation and importance of each soil properties were analyzed (Figure 4.1). The factors which locate closely can be determined to have similar effect on results, in Figure 4.1, EC (Electrical Conductivity) and soil texture seemed to have similar effect on soil microbial activity.

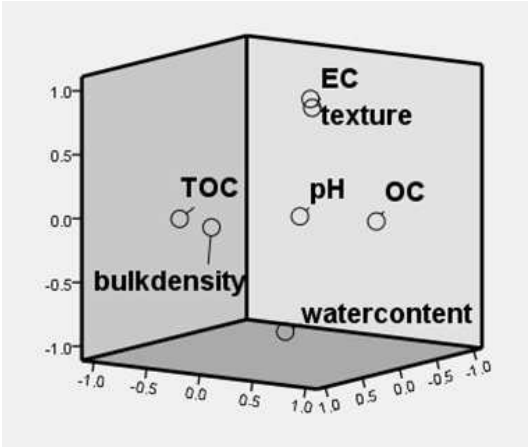


Figure 4.1. The result of factor analysis showing the similarity of effects of factors on microbial activity

The pH of the soil has found to cause most significant effect among other soil properties decided by statistical analysis. MANOVA (Multivariate Analysis of Varience) was used to compare several dependent variables, soil physical properties in this study. Multivariate F values were obtained only for different pH values and determined to have significant correlation with microbial activity test results, also indicating that other soil properties might not have statistically significant effect on soil microbial activity.

Since the amount of peat soil was not enough to analyze physical properties, experimental results of peat soil could not be used for statistical analysis, however, the other differences between peat soil and other soil types was observed indicating that with higher organic matter content in soil, the bioavailability can get lower.

4.1.1.1 Potential nitrification activity

The result was derived by analyzing the nitrite and nitrate concentration using IC. Nitrite concentration has increased and nitrate concentration has decreased by denitrification process. Potential nitrification activity method seemed to have low sensitivity measuring soil microbial activity. One of possible reasons was assumed that unlike other six microbial activity measurements potential nitrification activity needs ion concentration analysis, therefore with small amount of soil, measuring the concentration of nitrite and nitrate can be difficult regarding the detection limit of IC.

Table 4.1. Derived NOECs of different soil types and exposure times using potential nitrification activity tests (unit: mg/kg)

Soil type/Exposure time	2 weeks		4 weeks		8 weeks	
	TNT	RDX	TNT	RDX	TNT	RDX
Firing range soil	65	350	60	340	55	330
Field soil	55	300	50	275	50	250
Paddy soil	45	265	40	250	40	250
Landfill soil	55	300	50	275	50	275
Sand	50	300	50	275	45	275
Clay	55	330	50	315	50	310
Peat	60	330	55	320	55	320

4.1.1.2 Dehydrogenase activity

Dehydrogenase activity method has most frequently selected as soil microbial activity test method since most soil microbes participate in dehydrogenase activity. The existence of alive cell can be distinguished by several method such as basal respiration, substrate-induced respiration and dehydrogenase activity, and dehydrogenase activity method can be performed easily and accurately compared to basal respiration and substrate-induced respiration, however in this study, dehydrogenase activity was found to be least sensitive method to determine soil microbial activity changes.

Table 4.2. Derived NOECs of different soil types and exposure times using dehydrogenase activity tests (unit: mg/kg)

Soil type/Exposure time	2 weeks		4 weeks		8 weeks	
	TNT	RDX	TNT	RDX	TNT	RDX
Firing range soil	NA	NA	70	400	65	380
Field soil	60	320	50	300	50	290
Paddy soil	50	290	45	275	45	275
Landfill soil	60	320	50	300	50	290
Sand	55	290	50	275	50	275
Clay	60	320	50	320	50	300
Peat	75	350	65	320	65	320

4.1.1.3 Phosphatase activity

Phosphatase activity uses the hydrolysis characteristics of phosphatase which can indicate phosphatase activity method can be popular since hydrolysis occurs frequently among various microbial communities. Phosphate activity was found to be a sensitive method compared to other soil activity methods such as dehydrogenase activity, potential nitrification activity, fluorescein diacetate hydrolytic activity and rhodanese activity meaning that phosphate activity can be preferred to use for determination of small soil microbial activity differences.

Table 4.3. Derived NOECs of different soil types and exposure times using phosphatase activity tests (unit: mg/kg)

Soil type/Exposure time	2 weeks		4 weeks		8 weeks	
	TNT	RDX	TNT	RDX	TNT	RDX
Firing range soil	60	350	60	330	55	330
Field soil	45	275	40	250	35	225
Paddy soil	45	240	40	225	40	225
Landfill soil	55	320	50	315	50	315
Sand	50	275	45	250	40	250
Clay	50	315	45	300	45	295
Peat	60	330	55	325	50	325

4.1.1.4 Fluorescein diacetate hydrolytic activity

Most enzymes are known to participate in fluorescein diacetate hydrolysis process and produce fluorescein diacetate, therefore fluorescein diacetate hydrolytic activity can be widely used for assessing soil microbial activity. Fluorescein diacetate hydrolytic activity seemed to have low sensitivity measuring soil microbial activity in this study, however Fluorescein diacetate hydrolytic activity can be selected to measure total microbial activity since fungi, most bacteria, protozoa and algae can show fluorescein diacetate hydrolytic activity.

Table 4.4. Derived NOECs of different soil types and exposure times using fluorescein diacetate hydrolytic activity tests (unit: mg/kg)

Soil type/Exposure time	2 weeks		4 weeks		8 weeks	
	TNT	RDX	TNT	RDX	TNT	RDX
Firing range soil	NA	400	65	400	50	375
Field soil	55	300	50	275	50	275
Paddy soil	45	275	40	275	40	250
Landfill soil	55	300	55	280	55	275
Sand	50	300	45	300	45	250
Clay	50	325	45	315	40	315
Peat	65	350	60	325	60	325

4.1.1.5 β-glucosidase activity

The result indicated that β-glucosidase activity can be preferred to use for determination of small soil microbial activity difference since β-glucosidase activity was found to be a sensitive method compared to other soil activity methods such as dehydrogenase activity, potential nitrification activity, fluorescein diacetate hydrolytic activity and rhodanese activity. A wide distribution of β-glucosidase in nature and the dominance of β-glucosidase can provide fresh momentum to the fact that β-glucosidase activity can be regarded as a popular method.

Table 4.5. Derived NOECs of different soil types and exposure times using β-glucosidase activity tests (unit: mg/kg)

Soil type/Exposure time	2 weeks		4 weeks		8 weeks	
	TNT	RDX	TNT	RDX	TNT	RDX
Firing range soil	55	375	50	375	50	370
Field soil	45	275	40	260	40	260
Paddy soil	40	275	35	250	35	245
Landfill soil	55	325	45	300	45	300
Sand	50	300	45	275	40	250
Clay	50	325	45	325	40	300
Peat	55	345	50	340	50	325

4.1.1.6 Arylsulfatase activity

Arylsulfatase activity has used for soil microbial activity assessment method most frequently among sulfatases since arylsulfatase was first detected in nature compared to other sulfatases, however compared to other enzymes participate in other enzymatic reactions, arylsulfatase activity has not used widely relatively. Arylsulfatase activity was found to be most sensitive determined by the result of this study.

Table 4.6. Derived NOECs of different soil types and exposure times using arylsulfatase activity tests (unit: mg/kg)

Soil type/Exposure time	2 weeks		4 weeks		8 weeks	
	TNT	RDX	TNT	RDX	TNT	RDX
Firing range soil	60	375	55	350	55	350
Field soil	45	300	40	275	40	250
Paddy soil	40	250	35	225	35	225
Landfill soil	50	320	45	315	40	315
Sand	45	275	40	250	40	250
Clay	50	320	45	300	40	300
Peat	60	330	55	320	50	320

4.1.1.7 Rhodanese activity

Rhodanese activity has not used for soil microbial activity assessment method frequently. Rhodanese activity test seemed to have low sensitivity measuring soil microbial activity compared to other enzyme activity test methods.

Table 4.7. Derived NOECs of different soil types and exposure times using rhodanese activity tests (unit: mg/kg)

Soil type/Exposure time	2 weeks		4 weeks		8 weeks	
	TNT	RDX	TNT	RDX	TNT	RDX
Firing range soil	65	375	60	350	60	330
Field soil	50	275	45	250	40	250
Paddy soil	50	290	45	275	45	275
Landfill soil	50	300	45	290	45	290
Sand	45	275	45	275	45	275
Clay	50	325	50	315	40	315
Peat	50	330	50	315	50	315

4.1.2 Toxicity Endpoint Suggestion

NOECs vary between indicators, ranging from 37 to 65 for TNT and 225 to 400 for RDX (difference above 35%). Final NOECs were derived by calculating geometric mean of NOECs from different soil types for each indicators. Comparison of the derived NOECs of all indicators indicates that TNT is ecologically more toxic or more bioavailable than RDX. These ecotoxicological information would be useful in better defining the permissible concentration, criteria and reference values required for ecological risk assessments of explosive-contaminated sites.

Table 4.8. Final NOECs of TNT and RDX using seven different activity tests (unit: mg/kg)

	TNT	RDX
Potential nitrification activity	51.31	295.3
Dehydrogenase activity	55.15	308.9
Phosphatase activity	47.80	285.9
Fluorescein diacetate activity	45.31	301.8
β-glucosidase activity	50.21	306.1
Arylsulfatase activity	45.36	293.0
Rhodanese activity	48.45	297.9

4.2 Derivation of Ecologically Permissible Concentration

4.2.1 Ecologically Permissible Soil Concentrations using RIVM Approach

Dutch RIVM suggests the soil quality criteria derivation method using toxicity data. According to Dutch RIVM approach, chronic NOEC and chronic EC_{10} can only be used among other endpoints. NOEC is preferred than EC_{10}, however if toxicity data requirement cannot be satisfied using NOEC, EC_{10} is included for derivation process. Toxicity data requirement is that at least 10 chronic toxicity data (NOEC) from 10 species are needed in order to use species sensitivity distribution as data extrapolation method. Suggested ecologically permissible soil concentration can be calculated by the geometric mean of HC_5/UF and HC_{50}, which are derived from species sensitivity distribution and UF (uncertainty factor) decided by professional judgement from 1 to 5. No ecological concentrations are suggested for TNT and RDX by Dutch RIVM.

For TNT, species sensitivity distribution of each plant/invertebrate, wildlife and microbe were derived using terrestrial toxicity data. The species sensitivity distribution which showed most conservative HCx values were selected as a representative species sensitivity distribution which was plant/invertebrate species group in this study. The selected species sensitivity distribution of plant/invertebrate among derived species sensitivity distributions of each plant/invertebrate, wildlife, microbe is shown in Figure 4.2 (a). 12 NOECs from 10 plant/invertebrate species were collected after screening and qualification process. HC_5 values were 10.3, 18, 39 mg/kg and HC_{50} values were 30.7, 56.2, 114.8 mg/kg using

plant/invertebrate, wildlife, microbe, respectively. The suggested ecologically permissible soil concentrations of TNT are calculated and shown in Table 4.9.

For RDX, species sensitivity distribution of each plant/invertebrate and wildlife were also derived using terrestrial toxicity data. Species sensitivity distribution of microbe could not be derived since terrestrial toxicity data of RDX on microbe were not found. The selected species sensitivity distribution of plant/invertebrate between derived species sensitivity distributions of each plant/invertebrate and wildlife is shown in Figure 4.2 (b). 12 NOECs from 10 plant/invertebrate species were collected after screening and qualification process. HC_5 values were 23.7, 21.4 mg/kg and HC_{50} values were 71, 85 mg/kg using plant/invertebrate, wildlife respectively. The suggested ecologically permissible soil concentrations of RDX are calculated and shown in Table 4.9.

Table 4.9. Suggested ecologically permissible soil concentrations of TNT and RDX by Dutch RIVM approach using two different UF (uncertainty factors) (unit: mg/kg)

	Used uncertainty factor	TNT	RDX
Ecologically permissible soil concentration	UF=5 (conservative)	7.7	18.3
	UF=1	17.3	41

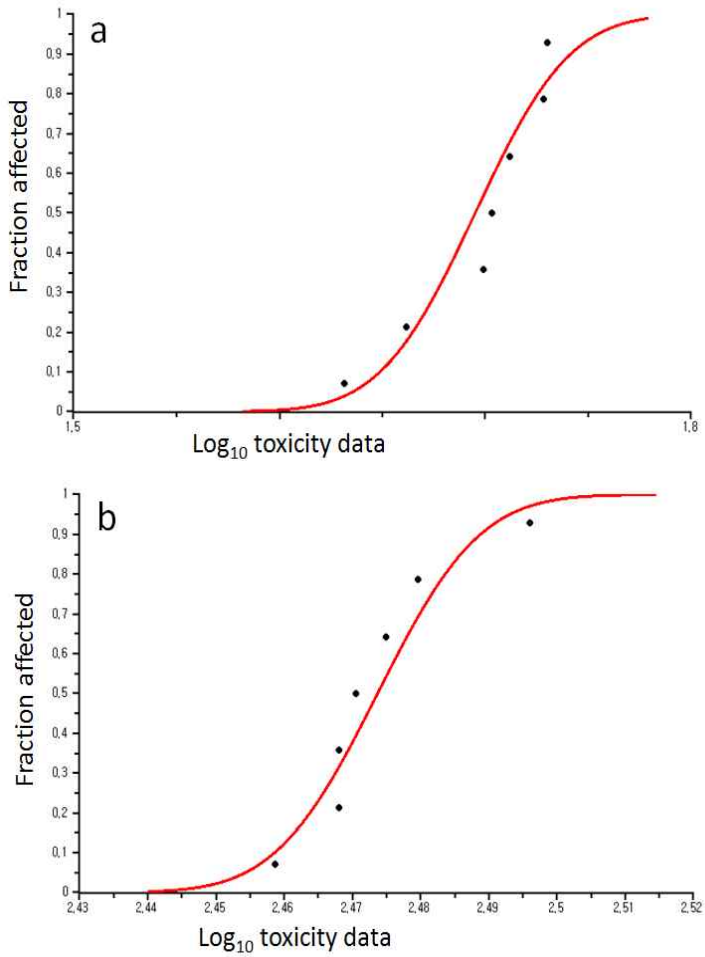


Figure 4.2. The SSD curve for derivation of ecologically permissible soil concentration of TNT (a) and RDX (b) using Dutch RIVM approach

4.2.2 Ecologically Permissible Soil Concentrations using CCME Approach

Canadian CCME suggests the protocol for deriving soil quality standards using toxicity data. Chronic ECx, LOEC and NOEC can only be used among other endpoints. ECx is preferred than LOEC and LOEC is preferred than NOEC. All chronic ECx, LOEC and NOEC can be used to satisfy toxicity data requirements but if the toxicity data requirements are satisfied, no further less preferred endpoints are allowed to use. Toxicity data requirement is that at least 10 chronic toxicity data from 3 different experiments are needed in order to use species sensitivity distribution as data extrapolation method. Suggested ecologically permissible soil concentration can be calculated by dividing HC25 by UF. SCMTS (Surface water standard for army suggested by Department of National Defense and Canadian Armed Forces) suggested soil quality standards of TNT as 9.6 and RDX as 10.8 mg/kg using Canadian CCME approach.

For TNT, species sensitivity distribution of each plant/invertebrate, wildlife and microbe were derived using terrestrial toxicity data. The species sensitivity distribution which showed most conservative HCx values were selected as a representative species sensitivity distribution which was plant/invertebrate species group in this study. The selected species sensitivity distribution of plant/invertebrate among derived species sensitivity distributions of each plant/invertebrate, wildlife, microbe is shown in Figure 4.3 (a). 24 toxicity data from 15 plant/invertebrate species were collected after screening and qualification process. HC_{25} values were 28.1, 56.2, 168 mg/kg using plant/invertebrate, wildlife, microbe, respectively. The suggested ecologically permissible soil concentrations of TNT are

calculated using two different UF and shown in Table 4.10.

For RDX, species sensitivity distribution of each plant/invertebrate and wildlife were also derived using terrestrial toxicity data. Species sensitivity distribution of microbe could not be derived since terrestrial toxicity data of RDX on microbe were not found. The selected species sensitivity distribution of wildlife between derived species sensitivity distributions of each plant/invertebrate and wildlife is shown in Figure 4.3 (b). 23 NOECs from 15 wildlife species were collected after screening and qualification process. HC_{25} values were 260, 75 mg/kg using plant/invertebrate, wildlife respectively. The suggested ecologically permissible soil concentrations of RDX are calculated and shown in Table 4.10.

Table 4.10. Suggested ecologically permissible soil concentrations of TNT and RDX by Canadian CCME approach using two different UF (uncertainty factors) (unit: mg/kg)

	Used uncertainty factor	TNT	RDX
Ecologically permissible soil concentration	UF=5 (conservative)	5.6	15
	UF=1	28.1	75
Soil quality standard suggested by SCMTS	UF value not stated	9.6	10.8

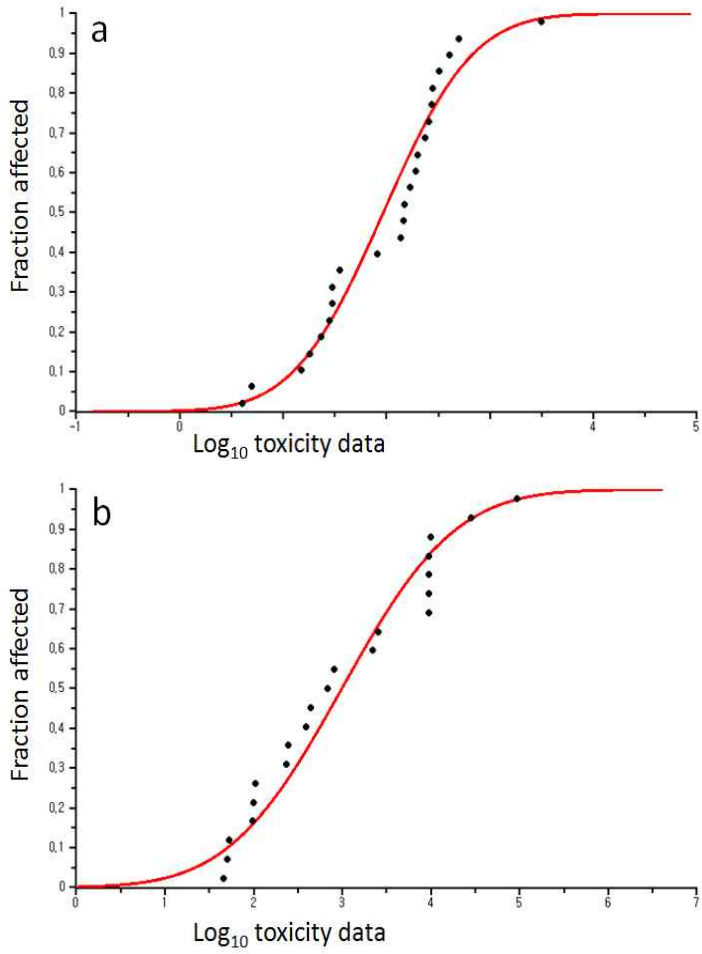


Figure 4.3. The SSD curve for derivation of ecologically permissible soil concentration of TNT (a) and RDX (b) using Canadian CCME approach

4.2.3 Comparison of Ecologically Permissible Soil Concentrations among Major Trophic Levels

Species sensitivity distributions were derived using microbial activity test results of this study (Figure 4.4). Unlike Canadian CCME or Dutch RIVM approach, all screened and qualified endpoints were used to derive species sensitivity distribution and HC_5 for protecting 95% of terrestrial species were selected. The HC_5 values of TNT, RDX are 43.02, 284.6 mg/kg and HC_{50} values of TNT, RDX are 49.85, 297.9 mg/kg, respectively. When comparing the ecologically permissible soil concentrations (Table 4.11) derived using different terrestrial species groups, plant/invertebrate group was found to be most sensitively affected by both TNT and RDX. Ecologically permissible soil concentration of TNT based on microbial activities were slightly different between 43.02 mg/kg based on microbial activity test results of this study and 30.6 mg/kg based on microbial test results gathered from other references. 43.02 mg/kg was derived using seven microbial activity test results, and 30.6 mg/kg was derived using nine microbial test results. Although more toxicity informations were used to derive 30.6 mg/kg, four out of nine endpoints were not microbial activity test results which were decided by measuring microbial biomass.

Table 4.11. Ecologically permissible soil concentrations derived using different species groups (unit: mg/kg)

	Species group used for derivation	TNT	RDX
Ecologically permissible soil concentration (HC_5 of SSD)	NOEC using microbial activity test results	43.02	284.6
	plant/invertebrate	3.4	3.1
	wildlife	15.2	21.4
	microbe	30.6	-

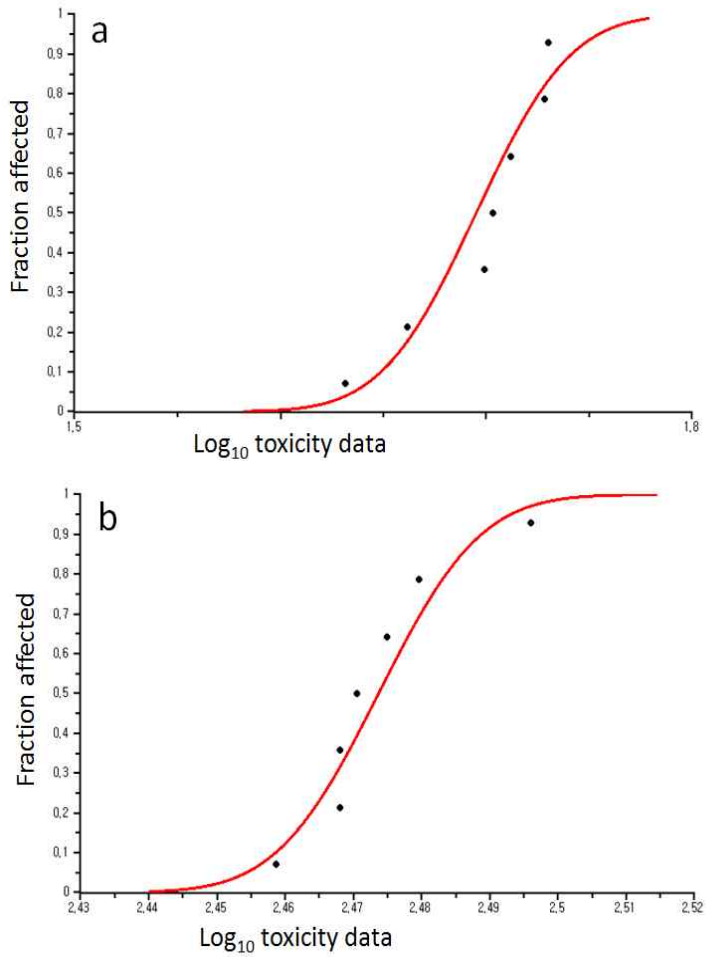


Figure 4.4. The SSD curve for derivation of ecologically permissible soil concentration of TNT (a) and RDX (b) using microbial activity test results of this study (NOEC)

4.3 Application in a Korean Active Firing Range

4.3.1 Site Characterization

Darakdae active firing range locates in Gyeonggi province, Korea. The area of the firing range is about 1.32 km^2 and explosives contamination occur all over the firing range. Since the firing range locates at elevation and Hantan river is located near firing range, the contaminants onsite can be transported to the river when heavy rainfall occurs. Surface runoff, ground runoff and soil erosion should be considered for assessing the transportation of explosives.

Various species of mammal, bird, fish and plants found at Darakdae active firing range and surrounding areas. For mammal, 26 species such as water deer, wild boar and squirrel, for bird, 77 species including barn swallow, wild goose and carrion crow, for plant, 90 species such as *Typha orientalis*, reed and willow and for fish especially, 370 endangered species including *Gobiobatia bervibarba* and Korean bitterling were found. Soil contamination with explosives in active firing ranges could be influential to the surrounding ecosystem, therefore, ecological receptors should be considered and risk assessment in active firing ranges should be conducted ecologically.

Contamination of TNT and RDX were inspected in Darakdae active firing range since 2009, however site inspections were not performed using incremental sampling. Explosives tend to contaminate the site partially, therefore detected concentration vary significantly by samples. Due to the contamination characteristics of explosives, incremental sampling should be used and executed in 2013 at Darakdae active firing range.

Incremental sampling should be conducted in active firing range since explosives contamination occurs locally. Incremental sampling was proposed by USACE, at least 30 incremental samples (1 incremental sample for every 25 m^2) should be collected in each unit area. Concentration of TNT and RDX differs significantly by the investigation methods, therefore this study used contaminant concentrations all from incremental sampling when assessing ecotoxicological risk assessment. Table 4.12 shows the results of incremental sampling including detection frequency, maximum concentration, representative concentration and arithmetic concentration.

One representative concentration of each TNT and RDX should be determined for ecotoxicological risk evaluation at a Korean active firing range. The representative concentration is set to 95% of the upper confidence limit on the arithmetic mean (UCL) according to USEPA (1989). ProUCL version 4.1 Software was selected for deriving the representative concentration in this study (ProUCL. 2010).

Table 4.12. Site inspection using incremental sampling (unit: mg/kg)

	TNT	RDX
Detection frequency	32/50 (64%)	50/50 (100%)
Maximum concentration	344.03	227.14
Representative concentration (95% ProUCL)	40.59	52.97
Arithmetic average concentration	0.43	22.48

4.3.2 Derivation of Ecologically Permissible Water Concentration

Aquatic toxicity data were collected from references, and screened using both Canadian CCME approach and Dutch RIVM approach. Screened toxicity data on aquatic species were used to derive a species sensitivity distribution. HC_5 value was chosen to suggest ecologically permissible water concentrations protectively. 89 toxicity data of 30 species on TNT and 45 toxicity data of 20 species on RDX were used for deriving species sensitivity distributions. Used toxicity data of both TNT and RDX included toxicity on fish, crustacean and algae. Derived HC_5 values are indicated in Table 4.13 using species sensitivity distribution shown in Figure 4.5.

Table 4.13. Ecologically permissible water concentrations of TNT and RDX (HC_5 of SSD) (unit: μg/L)

	TNT	RDX
Ecologically permissible water concentration	160	256

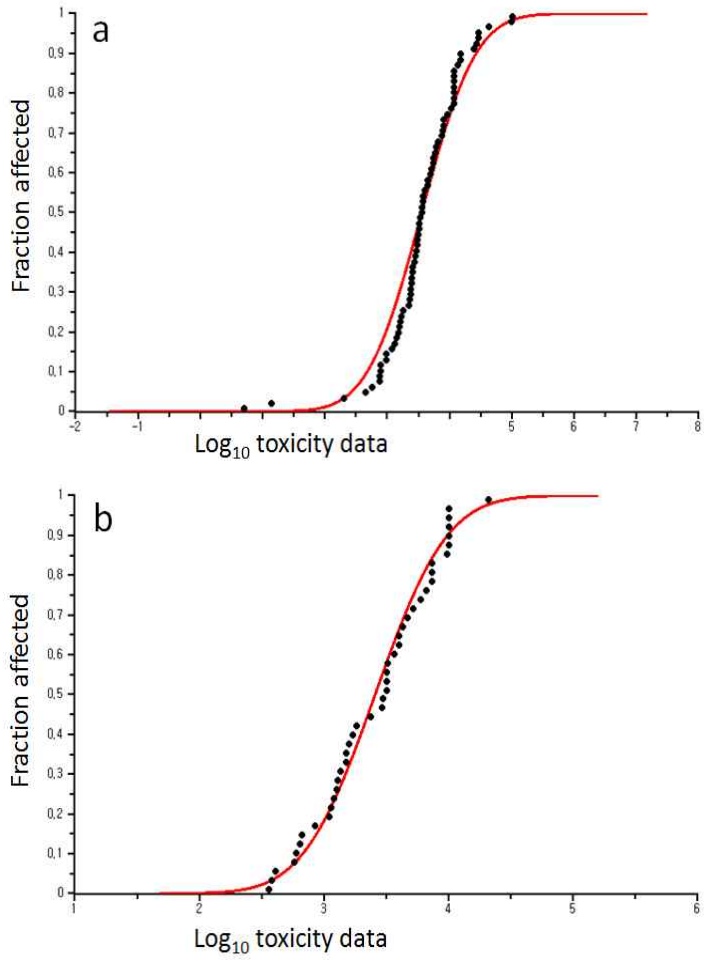


Figure 4.5. The SSD curve for derivation of ecologically permissible water concentrations of TNT (a) and RDX (b)

Table 4.14. Official water quality standards

Protocol	Standard type	Concentration (μg/L)	
		TNT	RDX
$SCMTS_E$	(Chronic)	120	190
R6	(Chronic)	100	360
ORNL	Chronic	130	190
	Acute	570	1400
Australia/NZ	(Chronic)	140	-

4.3.3 Derivation of Ecologically Permissible Firing Range Soil Concentration by Efflux Equation and Ecotoxicological Risk Assessment at Darakdae Active Firing Range

The efflux equation was derived using following soil screening level partitioning equation for migration to ground water (USEPA. 1996) using several assumptions (Table 4.15). Contaminant concentration in soil leachate from contaminants concentration absorbed to soil organic matter can be derived using the soil/water partitioning equation.

$$C_T = C_W[K_d + \frac{\theta_w + \theta_a H'}{\rho_b}]$$

Table 4.15. The assumptions used in the efflux equation

Assumptions
Runoff contaminants consist of dissolved (by runoff) and adsorbed (by sediment flux) contaminants
Concentration of dissolved contaminants are same with concentration of contaminants in soil pores
Concentration of adsorbed contaminants are same with concentration of contaminants adsorbed to soil particles
No other pathways considered such as decomposition of contaminants

$$C_T = \frac{C_W(K_d + \frac{\theta_W + \theta_a H'}{\rho_b})(Q_{sr} + Q_{gr} + Q_{up})}{(Q_{sr} + Q_{gr} + K_d Y)}$$

The efflux equation can explain the contaminant flux and concentration in ground water, surface water and sediment flux. There are chemical-specific parameters and site-specific parameters in the equation. For a better application of efflux equation in other TNT, RDX-contaminated sites, the sensitivity of site-specific parameters were analyzed.

The sensitivity of site-specific parameters such as corrosion coefficient, slope length-gradient coefficient, soil cover coefficient and soil density were determined, various values of each parameters were collected by literature review (Table 4.16). Sensitivity was calculated using minimum, median, maximum value, change one parameter at a time among minimum and maximum value when other parameters fixed to median value.

Table 4.16. The sensitivity of site-specific parameters

Parameter	Definition	Value	**Sensitivity**
Θ_w	Porosity in water	0.28	0.187
Θ_a	Porosity with air	0.16	0
ρ_b	Soil density	1.49	0.113
K	Erosion coefficient	0.22	0.536
LS	Slope factor	28.6	0.00113
C	Soil cover coefficient	0.013	0.0531
P	Soil control coefficient	1	0.0531
S_d	Reaching percentage	0.16	0.0531

Table 4.17. Parameters used in efflux equation

Parameter	Definition	Value	Unit	Reference
C_T	Ecologically permissible firing range soil concentration	-	mg/kg	-
C_W	Contaminant concentration in water	-	mg/L	this study
Q_{up}	Upflow flux	1.6E+08	m^3/month	NIER* (avg of 2008-2012)
Q_{sr}	Runoff efflux	6.785E+04	m^3/month	Simulated by SESOIL
Q_{gr}	Groundwater efflux	4.393E+05	m^3/month	
K_d	Adsorption coefficient	TNT-15.75 RDX-0.4988	L/kg	$K_{oc}*f_{oc}$
H'	Henry's constant	TNT-2.08E-08 RDX-2.01E-11	-	
Θ_w	Porosity in water	0.28	-	$n(1/K_s)^{1/(2b+3)}$
Θ_a	Porosity with air	0.16	-	$n-\Theta_w$
ρ_b	Soil density	1.49	kg/L	"D" report
Y	Sediment flux	1068	ton/month	$R_rKLSCPS_d$
R_r	Rainfall/leaching coefficient	205	10^7 J/ mm/ha	
K	Erosion coefficient	0.22	tons/ha/ R_r unit	"D" report
LS	Slope factor	28.6	-	
C	Soil cover coefficient	0.013	-	covered by grass(60%)
P	Soil control coefficient	1	-	no control
S_d	Reaching percentage	0.16	-	$(D_d)^{-0.22}$

4.3.4 Screening Level Ecological Risk Assessment

Screening level ecological risk can be determined by comparing predicted environmental concentration (PEC) and predicted no effect concentration (PNEC). PEC is a site-specific value and for Darakdae active firing range site incremental sampling was conducted for site contamination inspection. Representative concentrations (95% ProUCL) of detected concentrations at Darakdae firing range were used. Hazardous quotient (HQ) can be derived by dividing PEC with PNEC. By the value of PEC/PNEC or HQ, the existence of ecotoxicological risk can be determined, when HQ value is larger than 1, it is assumed that target contaminant might have ecotoxicological risk and further investigation or remediation is needed.

Table 4.18. Ecotoxicological risk assessment results (unit: mg/kg)

	PEC	PNEC	HQ
TNT	40.59	807	0.05
RDX	52.97	56	0.95

HQ values shown in Table 4.18 indicates that TNT and RDX have low possibility of affecting aquatic species dwelling in Hantan river. However, RDX might have ecotoxicological risk on aquatic species since the HQ value of RDX is close to one. At highly active firing ranges, direct soil remediation is impractical, therefore management of active firing range considering water quality is needed and controlling explosives on surrounding ecosystem, preventing transportation of explosives contamination can be more reasonable to manage the toxic effect of explosives.

5. Conclusion

This study showed that TNT and RDX have toxic effect on soil microbes by conducting soil microbial activity tests, and derived microbial NOECs from test results. In addition, derived NOECs were used for setting screening level ecological soil concentrations. Furthermore, this study suggested ecologically permissible soil concentrations of TNT and RDX by using species sensitivity distribution consists of collected toxicity data. Finally ecologically permissible concentrations were used for ecotoxicological risk assessment in active firing range in Korea.

(1) Microbial NOECs of TNT and RDX were derived by soil microbial activity tests using seven different soil types. Soil microbial activity decreased with increasing TNT and RDX concentration and with longer exposure time. Indigenous microbes were determined to be no longer functional or alive after 4 weeks of exposure in average since NOECs of different exposure time became constant after 4 weeks. Microbial NOECs of TNT and RDX were varied by soil pH when comparing paddy soil and field soil, low pH seemed to have additional toxic effect on soil microbes. soil texture when comparing clay and landfill soil to other soil types and organic content determined by peat soil having high soil microbial activity. Especially firing range indigenous microbes are found to have tolerance since soil microbial activity of firing range soil were high even with comparably low pH.

(2) Species sensitivity distribution was derived using soil microbial activity test results and ecologically permissible soil concentrations of TNT and RDX on soil microbes were suggested. Ecological receptors must be categorized to three, plant/invertebrate, wildlife and microbe when assessing ecotoxicity on terrestrial species since toxicity might reveal differently by the residue of receptors. The most conservative value from all ecologically permissible soil concentrations of plant/invertebrate, wildlife and microbe was selected for representative value. Ecologically permissible concentration of TNT was 43.02 mg/kg and that of RDX was 284.6 mg/kg when only soil microbial activity was considered. Soil microbes seemed to get less impact from TNT and RDX compared to plant/invertebrate (TNT-3.4, RDX-3.1 mg/kg) and wildlife (TNT-15.2, RDX-21.4 mg/kg) which were HC_5 of species sensitivity distribution, however it is important to include toxicity on soil microbes when deriving ecologically permissible soil concentrations. The results of this study can have a significant role since there are not enough microbial toxicity data available for TNT and there is no available microbial toxicity data for RDX.

(3) Ecologically permissible soil concentrations of TNT and RDX using species sensitivity distribution consists of toxicity data on plant/invertebrate, wildlife and microbe were derived. Soil quality standard derivation method of Dutch RIVM and Canadian CCME were used for calculating ecologically permissible soil concentrations in this study. The differences between two standard derivation approaches were the difference of used toxicity endpoints to derive species sensitivity distribution, the percentage of protecting ecological receptors and the application of UF (uncertainty factor) in the derivation process. When using UF of 5, conservative values were derived, by Dutch RIVM, ecologically permissible soil concentration of TNT was 7.7 mg/kg, and that of RDX was 18.3 mg/kg, in addition, by Canadian CCME approach, ecologically permissible soil concentration of TNT was 5.6 mg/kg and that of RDX was 15 mg/kg. Less conservative ecologically permissible concentration can be derived using UF of 1, by Dutch RIVM approach, ecologically permissible soil concentration of TNT was 17.3 mg/kg and that of RDX was 41 mg/kg, moreover by Canadian CCME method, ecologically permissible soil concentration of TNT was 28.1 mg/kg and that of RDX was 75 mg/kg. Derived values can be chosen according to different purposes and land uses and can be used as screening level. In addition, capability and application of remediation and management technology and other social or economical conditions should be considered when setting soil standards.

(4) Ecologically permissible water concentrations of TNT and RDX were derived using species sensitivity distribution consists of collected aquatic toxicity data. Considering efflux in Darakdae active firing range, ecologically permissible firing range soil concentrations were calculated based on ecologically permissible water concentrations. Screening level ecological risk was determined by comparing predicted environmental concentration (PEC) and predicted no effect concentration (PNEC), the hazardous quotient (HQ) can be calculated by dividing PEC with PNEC. The actual environmental concentration found in active firing range was set as PEC and ecologically permissible water concentrations were set as PNEC for each TNT and RDX. HQ of both TNT (0.05) and RDX (0.95) were lower than 1 indicating there is no ecological risk. Through screening level ecological risk assessment, Darakdae active firing range was determined to have no ecological risk caused by TNT and RDX.

References

Anderson JPE, Domsch KH, 1978. A physiological method for the quatitative measurement of microbial biomass in soils. Soil Biol Biochem, 10, 215-221.

Coelman P, Haanstra L. 1986. Short- and long-term effects of heavy metals on urease activity in soils. Biol Fertil Soils, 2, 213-218.

Drzyzga O, Gorontzy T, Schmidt A, Blotevogel KH. 1995. Toxicity of explosives and related compounds to the luminescent bacterium Vibrio fischeri NRRL-B-11177. Arch Environ Contam Toxicol, 28, 229-235.

Dunnett CW. 1955. A multiple comparison procedure for comparing several treatments with a control, Journal of the American Statistical Association, 50, 1096 - 1121.

Dunnett CW. 1964. New tables for multiple comparisons with a control, Biometrics, 20, 482 - 491.

FAM Verdonch, J Jaworska, CR Janssen, PA Vanrolleghem. 2002. Probabilistic ecological risk assessment framework for chemical substances. Proceedings International Conference on Integrated Assessment and Decision Support, 1, 144-149.

George SE, Huggins-Clark G, Brooks LR. 2001. Use of a Salmonella microsuspension bioassay to detect the mutagenicity of munitions compounds at low concentrations. Mutation Research, 490, 45-56.

Gong P. 1997. Dehydrogenase activity in soil: A comparison between the TTC and INT assay under their optimum conditions. Soil Biol Biochem. 29, 211-214.

Gong P, Siciliano SD, Greer CW, Paquet L, Hawari J, Sunahara GI. 1999. Effects and bioavailability of 2,4,6-trinitrotoluene in spiked and field contaminated soils to indigenous microorganisms. Environ Toxicol Chem. 18, 2681-2688.

Gong P, Hawari J, Thiboutot S, Ampleman G, Sunahara GI. 2000. Ecotoxicological effects of hexahydro-1,3,5-trinitro-1,3,5-triazine on soil microbial activities. Environ Toxicol Chem. 20, 947-951.

Green VS, Stott DE, Diack M. 2006. Assay for fluorescein diacetate hydrolytic activity: Optimization for soil samples. Soil Biol Biochem. 38, 693-701.

Gunda Reddy, Sundeep AM Chandra, James W Lish, Charles W Quails. 2000. Toxicity of 2,4,6-trinitrotoluene (TNT) in hispid cotton rats (Sigmodon hispidus): Hematological, biochemical, and pathological effects. International Journal of Toxicology, 19, 169-177.

Heinemeyer O, Insam H, Kaiser EA, Walenzik G. 1989. Soil microbial biomass and respiration measurements: An automated technique based on infra-red gas analysis. Plant and Soil, 116, 191-195.

Ingo Schmidt, Olav Sliekers, Markus Schmid, Inina Cirpus, Marc Strous, Eberhard Bock, J Gijs Kuenen, Mike SM Jetten. 2002. Aerobic and anaerobic ammonia oxidizing bacteria-competitors or natural partners?. FEMS Microbiology Ecology, 39, 175-181.

Jian-Shen, Ji Spain, Jalal Hawari. 2003, Phylogenetic and metabolic diversity of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)-trnsforming bacteria in strictly anaerobic mixed cultures enriched on RDX as nitrogen source. FEMS Microbiology Ecology, 46, 189-196.

Jordan N Smith, Jun Liu, Marina A Espino, George P Cobb. 2007. Age dependent acute oral toxicity of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and two anaerobic *N*-nitroso metabolites in deer mice (*Peromyscus maniculatus*). Chemosphere, 67, 2267-2273.

Liqun Xing, Hongling Liu, John P Giesy, Xiaowei Zhang, Hongxia Yu. 2011. Probabilistic ecological risk assessment for three chlorophenols in surface waters of China. Journal of environmental sciences. 24, 329-334.

Montague MD, Dawes EA. 1974. The survival of *Peptococcus prevotii* in relation to the adenylate energy charge. Journal of General Microbiology, 80, 291-299.

Ofelia Andrea Valdes-Rodriguez, Odilon Sanchez-Sanchez, Arturo Perez-Vazquez. 2011. Soil texture effects on the development of *Jatropha* seedlings-Mexican variety '*pinon manso*'. Biomass and Bioenergy, 35, 3529-3536.

RIVM (National Institute for Public Health and the Envionment), Guidance for the derivation of environmental risk limits within the framework of 'International and national environmental quality standards for substances in the Netherlands'. 2007. 601782001. Directorate for Chemicals, Waste and Radiation, Bilthoven.

Rossel D, Tarradellas J, Bitton G, Morel JL. 1997. Use of enzymes in soil ecotoxicology: A case for dehydrogenase and hydrolytic enzymes. In Tarradellas J, Bitton G, Rossel D, eds, Soil Ecotoxicology. CRC, Boca Raton, FL, USA, 179-206.

Samantha F. 2004. Soil screening values for use in UK ecological risk assessment. Office of Soil Quality and Protection. Bristol.

Schnurer J, Rosswall T. 1982. Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. Applied and Environmental Microbiology, 43, 1256-1261.

SERDP (National Research Council of Canada), Development of toxicity benchmarks and bioaccumulation data for N-based organic explosives for terrestrial plants and soil invertebrates. 2012. ER-1416.

Skujins J. 1976. Extracellular enzymes in soil. CRC Crit. Rev. Microbiol. 4, 383-421.

Steve D Siciliano, R Roy, CW Grrer. 2000, Reduction in denitrification activity in field soils exposed to long term contamination by 2,4,6-trinitrotoluene (TNT). FEMS Microbiology Ecology, 32, 61-68.

Tobias Frische. 2001. Screening for soil toxicity and mutagenicity using luminescent bacteria-A case study of the explosive 2,4,6-trinitritolueme (TNT). Ecotoxicology and Environmental Safety, 51, 133-144.

Tobias Frische. 2003. Soil microbial parameters and luminescent bacteria assays as indicators for in situ bioremediation of TNT-contaminated soils. Chemosphere, 50, 415-427.

Torstensson L. 1993. Ammonium oxidation, a rapid method to estimate potential nitrification in soils. In Torstensson L, ed, *Guidelines: Soil Biological Variables in Environmental Hazard Assessment*. Swedish Environmental Protection Agency, Solna, 40-47.
U.S. Environmental Protection Agency. 1985. Guidelines for deriving numerical national water quality criteria for the protection of aquatic organisms and their uses. PB85-227049. Office of Research and Development, Minnesota.

U.S. Environmental Protection Agency. 1996. Soil screening guidance: User's guide. In test Methods for Evaluating Solid Waste. 540/R-96/018. Office of Emergency and Remedial Response, Washington DC.

U.S. Environmental Protection Agency. 1997. Method 8330-Nitroaromatics and nitramines by high performance liquid chromatography (HPLC). In test Methods for Evaluating Solid Waste. SW-846 Update Ⅲ, Part 4:1 (B). Office of Solid Waste, Washington DC.

Weixin Cheng and David C Coleman. 1988. A simple method for measuring CO2 in a continuous air-flow system: modifications to the substrate-induced respiration technique. Soil Biol Biochem, 21, 385-388.

Wheeler JR, Grist EP, Leung KY, Morritt D, Crane M, 2002. Species sensitivity distributions: data and model choice. Mar Pollut Bull. 45, 192-202.

Witter E, Kanal A. 1998. Characteristic of the soil microbial biomass in soils from a long-term field experiment with different levels of C input. Appl Soil Ecol. 10, 37-49.

초 록

토양미생물의 활성을 이용한 화약류의 토양 생태독성 및 생태학적 허용농도 결정에 관한 연구

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사격장 오염 토양에 대한 관심이 커지고 있으나 아직까지 화약물질에 대한 국내 법규와 정화기준이 존재하지 않아 화약물질을 정화 및 관리하기에 많은 어려움을 겪고 있다. 사격장 내 오염물질은 화약물질 뿐 아니라 중금속도 발견되나, 중금속의 경우 국내 법규 상 토양 기준이 대부분 존재하며, 중금속의 생태독성학적 영향에 관련하여 이미 많은 연구가 진행되었기 때문에 본 연구에서는 사격장 오염 부지의 주요 오염물질 중에서 화약물질을 선정하였다. 화약물질은 대표적으로 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), 1,3,5,7-tetranitro-1,3,5,7-tetrazocane (HMX) 이나 국내에서 HMX는 사용하지 않기 때문에 국내 실정에 맞는 TNT와 RDX를 대상 물질로 선정하였다.

다양한 생태수용체를 대상으로 한 독성평가 결과로 도출된 독성종말점은 생태학적 토양 허용농도를 도출하는 과정에서 사용된다. 하지만 미생물활성실험을 통한 토양미생물에 대한 독성영향을 평가한 독성자료가 부족한 실정이다. TNT의 경우 토양미생물에 대한 독성 종말점이 존재하나, 그중 일부는 미생물의 생체중량을 평가한 것으로 토양미생물 활성평가를 통한 독성자료의 수가 부족하며, RDX의 경우 토양미

생물을 대상으로 한 독성영향 평가가 거의 진행되지 않았다. 따라서 본 연구에서는 토양미생물 활성평가를 통하여 차후 생태독성학적 영향 평가에 이용될 수 있는 독성자료를 제시한다. 토양미생물 활성평가를 위해 potential nitrification activity, dehydragenase activity, phosphatase activity, fluorescein diacetate activity, β-glucosidase activity, arylsulfatase activity and rhodanese activity, 7가지의 효소반응을 2주, 4주 그리고 8주의 노출기간을 두어 측정하였다. 이때 다양한 토양 특성에 따른 독성발현의 정도 변화를 알아보기 위하여 사격장토양, 임야토양, 논토양, 매립지토양, 모래, 점토, 유기물을 이용하여 실험하였다. 각 토양별, 그리고 효소반응별 실험결과에서 NOEC (No Observed Effect Concentration)을 도출한 후 하나의 효소반응에 대한 대표적 NOEC 값을 기하평균으로 계산하였다. TNT의 경우, NOEC이 45.31 (fluorescein diacetate activity)에서 55.15 (dehydrogenase activity) mg/kg에 해당하는 값을 보였고 RDX의 경우, NOEC 값이 285.9 (phosphatase activity)에서 308.9 (dehydrogenase activity) mg/kg에 해당하였다.

최근 국제적으로 생태 수용체에 대한 고려가 점차 중요해지고 있다. 토양매체의 경우 토양 생태 중심의 위해성 평가와 인체 중심의 위해성 평가 모두를 고려하여 토양 오염을 관리하는 미국, 캐나다, 네델란드, 독일 등의 국가들과 달리 우리나라에서는 아직 인체 중심의 위해성 평가만 이루어졌을 뿐 생태 수용체에 대한 고려가 없는 실정이다.

국내 서식종, 국제 표준 시험종, 국제 서식종을 포함하여 TNT와 RDX의 토양 생물에 대한 독성 지표값 350여개를 미국의 EPA (Environmental Protection Agency), 캐나다의 CCME (Canadian Council of Ministers of the Environment) 와 논문을 통해 조사하였고, 수집된 자료는 qualification 과정을 통하여 신뢰도가 높은 자료만을 선별하였다. 독성자료를 도출하는 과정에서 국제표준 독성실험법을 사용하여야 하고, 노출기간, 독성종말점, 통계학적 처리방법, 인공오염 후 실제 오염농도 그리고 그 밖의 실험 조건에 대한 정확한 명시가 되어 있어야 한

다. 토양 독성 자료의 경우 토양 내 유기물 함량에 따라 독성 발현이 달라질 수 있으므로 기준 유기물함량에 맞도록 표준화 과정을 거쳤다. 조건을 만족하는 독성자료들을 기하평균을 통해 하나의 종과 하나의 독성 종말점에 대한 대표값을 계산하였다. 수집된 독성자료의 quality에 따라 종민감도분포도(SSD: Species Sensitivity Distribution), Assessment Factor method (AF), 그리고 Equilibrium Partitioning method 중 하나의 방법을 선정하였다.

어떤 물질의 환경기준을 결정하는 데에는 그 물질의 독성뿐 아니라 배경농도, 가용한 정화기술, 사회경제적 고려 등이 포함되어야 하지만, 본 연구에서 도출된 TNT와 RDX에 토양 생물이 5% 영향을 받는 농도인 HC_5는 생태 보호를 위한 토양의 TNT, RDX 관리기준의 기본 값으로는 사용될 수 있을 것이다.

사격장의 경우, 사람의 접근은 제한되어 있으나 주변 서식 동식물의 접근은 가능하기 때문에 토양 생태 수용체를 반드시 고려해야한다. 실제 국내 다락대 사격장과 주변지역을 조사한 결과, 고라니, 멧돼지, 다람쥐를 포함한 26 종의 포유류, 제비, 거위, 까마귀를 포함한 77 종의 조류, 부들, 갈대, 버드나무를 포함한 90 종의 식물이 발견되어 생태 수용체 고려의 필요성을 확인하였다.

본 연구에서 도출한 HC_5를 PNEC (Predicted No Effect Concentration)로 사용하고 한탄강 유역에 있는 다락대 종합사격장 TNT, RDX의 토양 오염 농도를 PEC (Predicted Exposure Concentration)으로 하여 HQ (Hazard Quotient)를 도출하여 생태 위해 여부를 판단하여 보았다. 토양 오염 농도인 PEC은 다락대 종합사격장 내 실시한 incermental sampling 결과, 발견농도의 대표농도 TNT-40.59 mg/kg (95% Gamma UCL), RDX-52.97 mg/kg (95% chebyshev UCL)을 사용하였다. PNEC의 경우 본 연구에서 도출한 다락대 사격장 주변 한탄강의 수생태계를 보호할 수 있는 생태학적 허용농도인 TNT-807 mg/kg, RDX-56 mg/kg을 사용하였다. 토양에서 TNT의 HQ는 대표 농도에서 0.05, RDX의 경우 0.95이므로 1 이하 값이 산출되어 생태학적으

로 위해 가능성이 없는 것으로 판단되었다. 도출한 HQ 값은 다락대 사격장 피탄지 토양에 존재하는 TNT와 RDX가 주변 수계인 한탄강의 수계생태계에 영향을 미칠 가능성이 없는 것을 의미하나, RDX의 경우 0.95이므로 추가적인 피탄지 토양에 대한 조사가 필요할 것으로 판단된다.

주요어 : TNT, RDX, 생태독성학적 허용농도, 토양 미생물 활성 평가, 생태독성학적 위해성평가

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