Guidance on the Assessment and Monitoring of Natural Attenuation of Contaminants in Groundwater

April 2024

CL:AIRE

ISBN: 978-1-905046-42-3 © CL:AIRE 2024

Published by CL:AIRE, Reading Business Centre, Fountain House, Queens Walk, Reading, RG1 7QF. Email: enquiries@claire.co.uk

Acknowledgements
This guidance has been prepared by a collaborative working group incorporating representatives from industry, consultancy, and regulators across the devolved administrations. CL:AIRE would like to gratefully acknowledge the Environment Agency for its seed funding for this project and the individuals of the working group for their time in authoring sections along with their technical discussions and review of the guidance. The final document has kindly been reviewed and supported by the Environment Agency, Natural Resources Wales, Scottish Environment Protection Agency and the Northern Ireland Environment Agency (NIEA), an agency within the Department of Agriculture, Environment and Rural Affairs (DAERA). CL:AIRE would also like to thank its Technology and Research Group for their peer review of the document prior to publication.

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Report Citation
It is recommended citation to this report is made as follows:

CL:AIRE, 2024. Guidance on the Assessment and Monitoring of Natural Attenuation of Contaminants in Groundwater. CL:AIRE, Reading. ISBN 978-1-905046-42-3. Download at www.claire.co.uk/mna

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Executive Summary

Monitored natural attenuation (MNA) can be a sustainable risk management strategy for a wide range of groundwater contaminants, where environmental data are collected and assessed that demonstrate natural attenuation will protect receptors from pollution or harm. Natural attenuation refers to the combination of physical, chemical and biological processes that act, without human intervention, to reduce contaminant concentrations, flux or toxicity. Natural attenuation of groundwater contaminants has been extensively researched over more than four decades. MNA therefore has a long track record of applications in the UK and elsewhere, either as the sole or primary remediation strategy, or the final stage following transition from active remediation.

The Environment Agency originally published technical guidance for MNA in 2000 in its R&D Publication 95. Since then, significant scientific advances have been made in understanding contaminant behaviour and reactive transport in the subsurface, alongside ongoing developments in site characterisation, monitoring and predictive modelling approaches and technologies, that are captured in this updated guidance. These evolving methods enhance contaminant and process-specific understanding, required to develop advanced conceptual site models for MNA, addressing complexities and uncertainties that were previously challenging to deal with. These advancements further support the development of three lines of evidence typically considered to demonstrate the effectiveness of natural attenuation for risk management in groundwater:

- Primary – reduction in contaminant concentration, mass and/or mass discharge in groundwater;
- Secondary – geochemical data and modelling that provides indirect evidence of the natural attenuation processes likely causing the observed reductions in contamination (primary line of evidence); and
- Tertiary – contaminant and/or process-specific evidence (e.g. isotopic, microbiological) to support the primary and secondary lines of evidence.

MNA viability is considered during remediation options appraisal. The phased approach described in this guidance supports identification of contaminant plumes for which MNA is likely feasible, then demonstrates the ability of natural attenuation to protect receptors now and in the future, and prior to undertaking a monitoring programme to confirm MNA will achieve remedial objectives within a timeframe suitable for all stakeholders:

- Step 1: MNA Screening – assessing feasibility against technical, practical, economic, sustainability and regulatory controls;
- Step 2: Field Demonstration – to provide evidence that natural attenuation is occurring and protective of receptors;
- Step 3: Predictive Modelling – to assure natural attenuation will remain effective in the future, considering potential adverse effects of changing conditions; and
- Step 4: Implementation of Performance Monitoring and Verification – a programme of groundwater monitoring confirming progress towards and ultimately meeting remedial objectives.

Understanding natural attenuation and performing MNA requires evolution of an advanced conceptual site model for relevant processes and associated risks that relies on quality data collection and analysis.

MNA represents a long-term commitment to groundwater risk management in the order of years to decades. If circumstances change, and MNA is no longer protective of receptors or viable to all stakeholders, then contingency measures may be required, including consideration of remediation alternatives to MNA. Engagement with the regulator is required throughout the decision-making process regarding MNA as a risk-management strategy for contaminated groundwater.

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A ‘Lines of Evidence’ Approach to Assessing Natural Attenuation

Natural attenuation (NA) processes act, without human intervention, to reduce the concentration, flux or toxicity of contaminants in soil and groundwater. Used as a remediation approach, Monitored Natural Attenuation (MNA) has a long track record of research and practical application in the UK and elsewhere.

This document provides guidance for practitioners on the science and practical aspects of implementing MNA in the UK. It is based on, and supersedes, Environment Agency R&D Publication 95 (Environment Agency, 2000).

Since the publication of the Environment Agency’s R&D Publication 95, a large amount of research has been undertaken on NA processes, new monitoring and assessment methods developed, and site-specific MNA projects completed, which are reflected in this updated document.

Natural attenuation (NA):
The effect of naturally occurring physical, chemical and biological processes, or any combination of those processes to reduce the concentration, flux or toxicity of substances in groundwater.

Monitored Natural Attenuation (MNA):
A risk-management approach that relies on monitoring of groundwater and technical evaluation to confirm whether NA processes are acting at a sufficient rate to ensure that unacceptable risks are managed.

A ‘lines of evidence’ approach is recommended for MNA assessment and verification. This evaluation normally fits within a broader site assessment and management strategy, following established land contamination risk-assessment and management processes (Environment Agency, 2023). An MNA lines of evidence assessment should build on an existing conceptual site model (CSM) and further describe the relevant NA processes and their effectiveness for relevant constituents of potential concern (CoPC). Three lines of evidence are typically considered (Rivett and Thornton, 2008):

- Primary – groundwater monitoring data that shows contaminant concentration, flux or toxicity decreases;
- Secondary – geochemical data and modelling that provides evidence of the process(es) causing the decreases (e.g. electron acceptor/donor data; aquifer geochemistry); and
- Tertiary – contaminant and process-specific (e.g. microbiological) evidence to support the primary and secondary lines of evidence.

MNA may achieve the remediation objectives when applied in isolation or may be used in combination with other remediation techniques. In the second situation a process-based technique is typically used to remove significant contaminant mass, and MNA applied as a secondary polishing step.

Consideration of MNA is likely to occur at two points during management of land contamination (e.g. Land Contamination Risk Management [LCRM] in England & Wales):

1. An assessment of MNA alongside other potential remediation options (e.g. Options Appraisal); and
2. Once selected as the preferred solution in the remediation options appraisal, a more detailed assessment of MNA using a lines of evidence approach is needed to demonstrate its effectiveness.

This guidance describes a phased approach. The first phase – MNA Screening – is the typical assessment necessary to support a remediation options appraisal. Once selected as the preferred risk-management solution, the additional three phases are followed as part of site-specific assessment and implementation.

In selecting any remediation solution, including MNA, it is recommended that the Options Appraisal includes assessment of the relative sustainability of feasible remediation options, for example, by using the SuRF-UK framework (CL:AIRE, 2010a).

The overall process for MNA assessment is illustrated in Figure 1.

A related concept, Natural Source Zone Depletion (NSZD), has also been introduced, which considers similar intrinsic processes in the depletion of light non-aqueous phase liquid (LNAPL) sources (Garg *et al.*, 2017; CL:AIRE, 2024). Whilst NA is generally focused on attenuation processes acting on dissolved phase compounds in groundwater and monitoring relies on groundwater sampling, NSZD largely focuses on processes acting on a NAPL source, and monitoring relies on carbon dioxide (CO_2) and methane (CH_4) fluxes in the unsaturated zone and proxies for biodegradation, such as temperature changes. The monitoring approaches for MNA and NSZD are distinct. Whilst assessment of NSZD is encouraged where appropriate, this document is restricted to MNA.

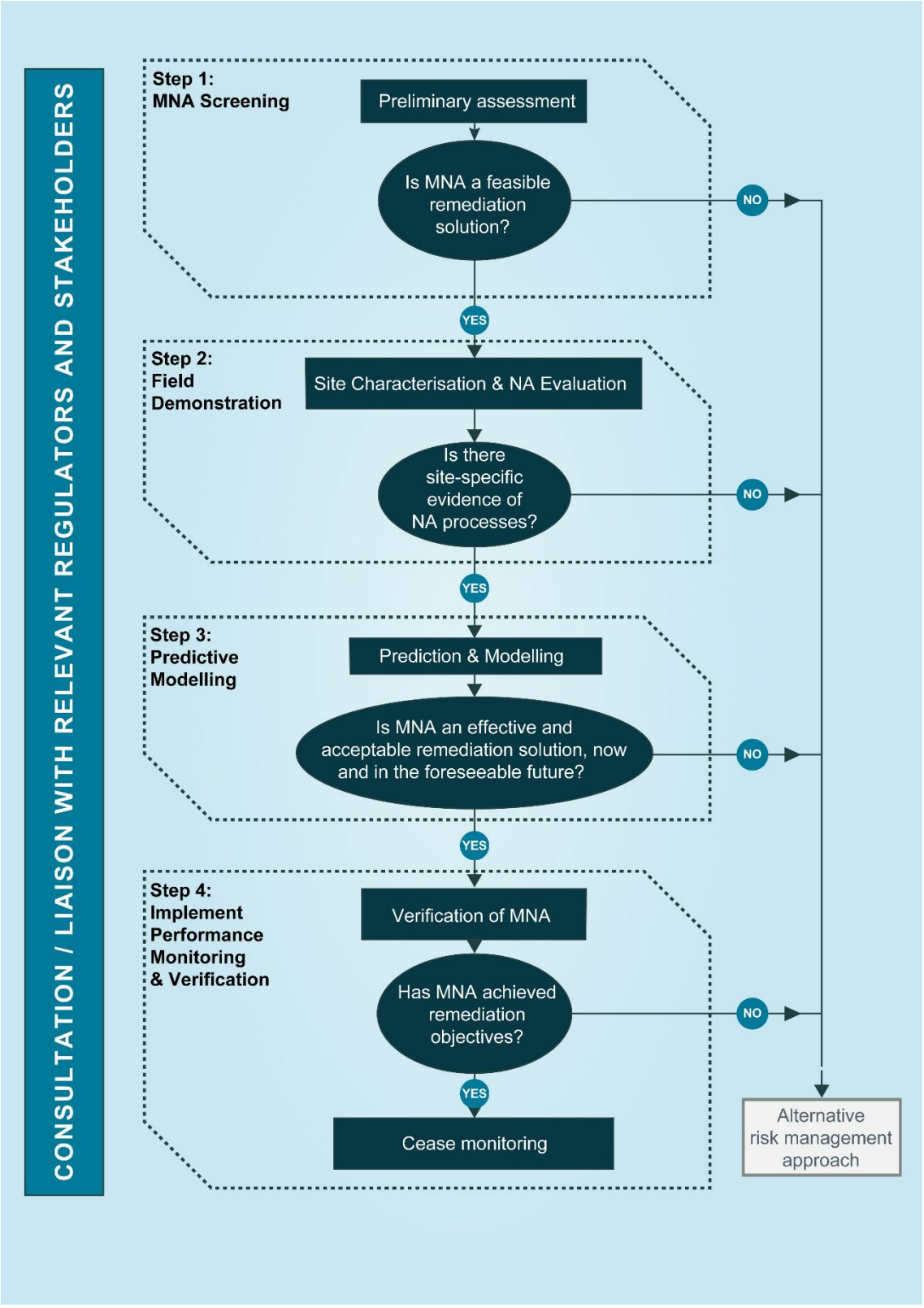


Figure 1: Step-wise approach to MNA assessment, showing main objectives and outputs at each step. The diagram shows a linear process, but there may be iteration.

Step 1: Monitored Natural Attenuation Screening

The first step in assessing MNA as a potential remediation option is to consider whether it is likely to be a feasible and effective remediation solution, which mitigates risks identified in the CSM and risk assessment process. The initial step is a desk-based assessment of the evidence to support MNA for the particular CoPC present. The assessment should consider:

- *Technical reliability*. Are NA processes likely to be effective in managing risks for all relevant source-pathway-receptor linkages throughout the duration of MNA?
- *Practicability*. Is there available time, access to areas of interest on the site and its surrounding, and access to monitoring points to implement an MNA strategy?
- *Economics*. Is MNA likely cost-competitive (considering whole-life costs) with other feasible options?
- *Sustainability*. Is MNA likely to be more sustainable than other feasible remediation options, when assessed against the broad sustainability criteria described by SuRF-UK (CL:AIRE, 2010a)?
- *Regulatory and Institutional Controls*. Is it compliant with the law and can risks be adequately controlled throughout the project duration?

When assessing technical reliability, assessors will find that some CoPC have a much larger research literature on NA processes, and published case studies on MNA implementation (see Appendix 2). For other CoPC the literature may be less comprehensive and there may be less evidence for prior investigation of NA processes. The approach to Step 1 should reflect the existing body of scientific research; for well-studied CoPC there may be no need for further assessment of the potential for biodegradation / attenuation at Step 1 other than to provide reference(s) to the published work.

In the case of MNA, which can take a number of years or even decades to complete, it is important that the sustainability assessment follows a holistic approach, such as that described by SuRF-UK. This particularly includes consideration of:

- SuRF-UK Indicator SOC 2 ‘Ethics and equity’, and in particular the effects on intergenerational equity by bequeathing unacceptable risk to future generations that might feasibly be addressed sooner; and
- SuRF-UK Indicator ECON 5 ‘Lifespan and flexibility’, and in particular the ability of an MNA strategy to manage risks in the long term where land-use or ownership might reasonably be expected to change within the duration of an MNA project, and/or be impacted by the potential effects of climate change (CL:AIRE, 2022; Environment Agency, 2023).

NA screening criteria covering a range of relevant considerations are presented in Appendix 1, and it is recommended that this structure forms the basis for an initial assessment of MNA viability.

A step-wise approach to MNA screening is provided in Figure 2.

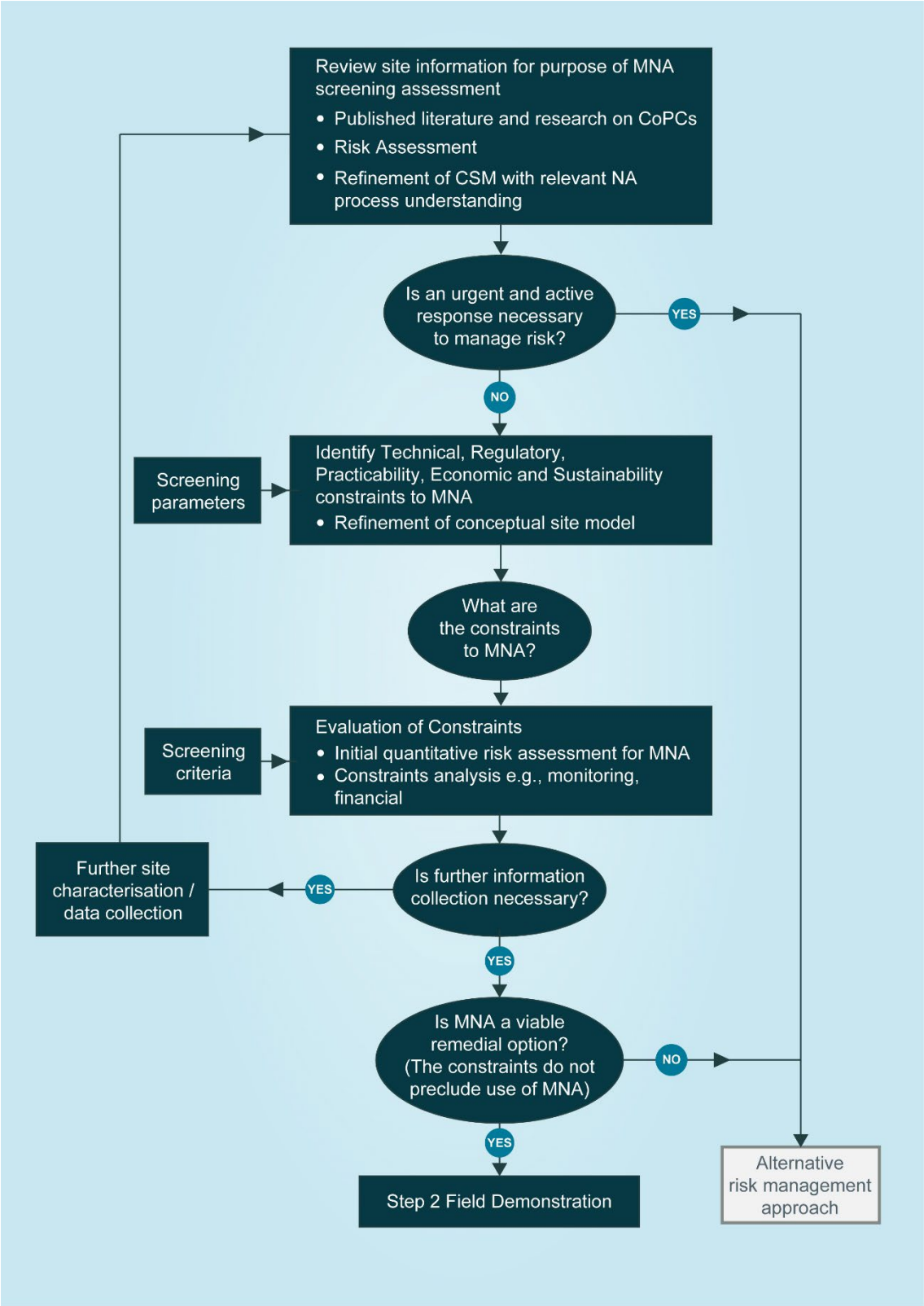


Figure 2: Step 1 – monitored natural attenuation screening.

Step 2: Field Natural Attenuation Demonstration

Having confirmed that NA processes are likely to occur at the site and that there are no obvious barriers to selecting an MNA strategy, Step 2 requires site-specific groundwater/aquifer physical and biogeochemical characterisation to demonstrate:

- whether the NA processes are currently occurring under site-specific field conditions;
- the rate at which NA processes occur;
- whether receptors are currently protected and identified risks are managed; and
- if receptors are not protected, why this might be, and what actions could be taken to enhance NA processes to overcome the limiting factors.

A lines of evidence approach is taken, and the results are often presented in graphical, statistical and/or visual form, and may be compared to predictive models (Appendix 5).

Collection of good quality environmental data that meets relevant data quality objectives, from a suitably designed and constructed monitoring network, is critical to ensure later interpretation of trends in contaminant concentration, mass discharge and behaviour is reliable. Characterisation data for MNA assessment should be incorporated into a refined CSM.

Monitoring of the concentrations and mass flux of the CoPC in relevant and consistent locations over time, and analysis of trends (to show sufficiently declining plume concentrations, mass discharge and stability or shrinkage) are the main requirements of the primary line of evidence. Supporting secondary evidence is provided by analysis of degradation products/metabolites, other compounds that are consumed or produced during the degradation of the CoPC (e.g. terminal electron acceptors, or electron donors), and geochemical parameters that influence attenuation potential including aquifer organic carbon content (f_{OC}), redox potential, and pH. Statistical analysis and visualisation can be adopted using spatial-temporal trend analysis and smoothing techniques, such as GWSDAT and MAROS, which can generate visual images of plume development over time.

Tertiary evidence includes microbiological and advanced geochemical data that can further indicate the extent of mineralisation, occurrence of microbial degradation processes, or other reactive processes, and also provide quantitative estimates of biodegradation rates.

In all instances the primary and secondary lines of evidence are needed. If these two lines of evidence are consistent and compelling the tertiary line of evidence may not be necessary, but if there is ambiguity the tertiary data may be helpful.

Where the evidence is weak or conflicting, or there are novel, unusual, or multiple CoPC that are subject to different NA processes, all three lines of evidence may be necessary. Table 1 illustrates typical lines of evidence data for common CoPC.

Table 1: Typical lines of evidence data requirements for common CoPC.

CoPC	Primary	Secondary	Tertiary
Petroleum hydrocarbons	Main CoPC, e.g. benzene, toluene, ethylbenzene, xylene (BTEX), total petroleum hydrocarbon fractions	Oxidants depleted (e.g. O_2 [dissolved oxygen], NO_3^- [nitrate], SO_4^{2-} [sulfate]) /by-products generated (e.g. Fe^{2+} [ferrous iron], Mn^{2+} [manganese II], S^{2-} [sulfide], CO_2, CH_4) during oxidation of CoPC pH, redox potential Inhibitory conditions (e.g. salinity)	Rarely necessary Microbial community analysis (e.g. cell counts) demonstrating increased biomass production within plume Compound specific isotope analysis (CSIA) of CoPC and/or electron acceptors
Chlorinated ethenes	Parent compound and contaminative degradation intermediates (e.g. tetrachloroethene, trichloroethene, cis-1,2-dichloroethene, vinyl chloride)	Degradation products, e.g. cis-1,2-dichloroethene, vinyl chloride, ethene, ethane, acetylene, Cl^- Electron donors – available (labile) organic carbon, H_2 [hydrogen] pH, redox potential Reactive FeS [iron sulfide] mineralogy Inhibitory conditions (e.g. O_2 [for highly saturated chlorinated compounds], SO_4^{2-}, chloroform)	Microbial species capable of reductive dechlorination e.g. *dehalococcoides* CSIA of CoPC
Ether oxygenates (either as pure product, or within gasoline)	Main CoPC, e.g. methyl tertiary-butyl ether (MTBE), tertiary amyl methyl ether (TAME), ethyl tertiary-butyl ether (ETBE)	Oxidants depleted in oxidation of CoPC, e.g. O_2, NO_3^-, SO_4^{2-}, or produced by redox process: Fe^{2+}, Mn^{2+}, S^{2-}, CO_2, CH_4 Degradation products, e.g. tert-butyl alcohol (TBA), tert-butyl formate (TBF) pH, redox potential	Rarely necessary if degradation products can be detected Gene sequencing for known ether oxygenates degradation potential, e.g. *Eth*-B

CoPC	Primary	Secondary	Tertiary
Ammonium	Ammonium	Oxidants depleted in aerobic or anaerobic oxidation of CoPC, e.g. O_2, NO_2^- [nitrite] Transformation products, e.g. NO_2^- , NO_3^-, N_2 [dissolved nitrogen] and N-oxide gases Aquifer cation exchange capacity (CEC), and dissolved major cations pH, redox potential	Rarely necessary
Metals	Metals Radioisotopes (if applicable)	Metals speciation Sorption coefficient, e.g. Kd CEC Mineralised forms (e.g. sulfides, oxides, carbonates etc) Major ions, redox chemistry pH, redox potential, Fe^{2+}, S^{2-}, TDS [total dissolved solids], DIC [dissolved inorganic carbon] and alkalinity	Stable isotope analysis

Guidance on biogeochemical assessment tools (tertiary line of evidence) is presented in Appendix 8 and Appendix 9.

Alongside specific and targeted data analyses described in Appendix 5, flow and transport models can be used to integrate and consider variability in complex site datasets to support demonstration that NA is effective. Modelling can provide a means to confirm the conceptual model for NA (i.e. whether simulation of the conceptual model matches observation data) and a rigorous framework for identifying data gaps and uncertainties. Modelling can be used to quantify attenuation processes and understand how current conditions arose and may change (Appendix 7).

A step-wise approach to field NA demonstration is provided in Figure 3.

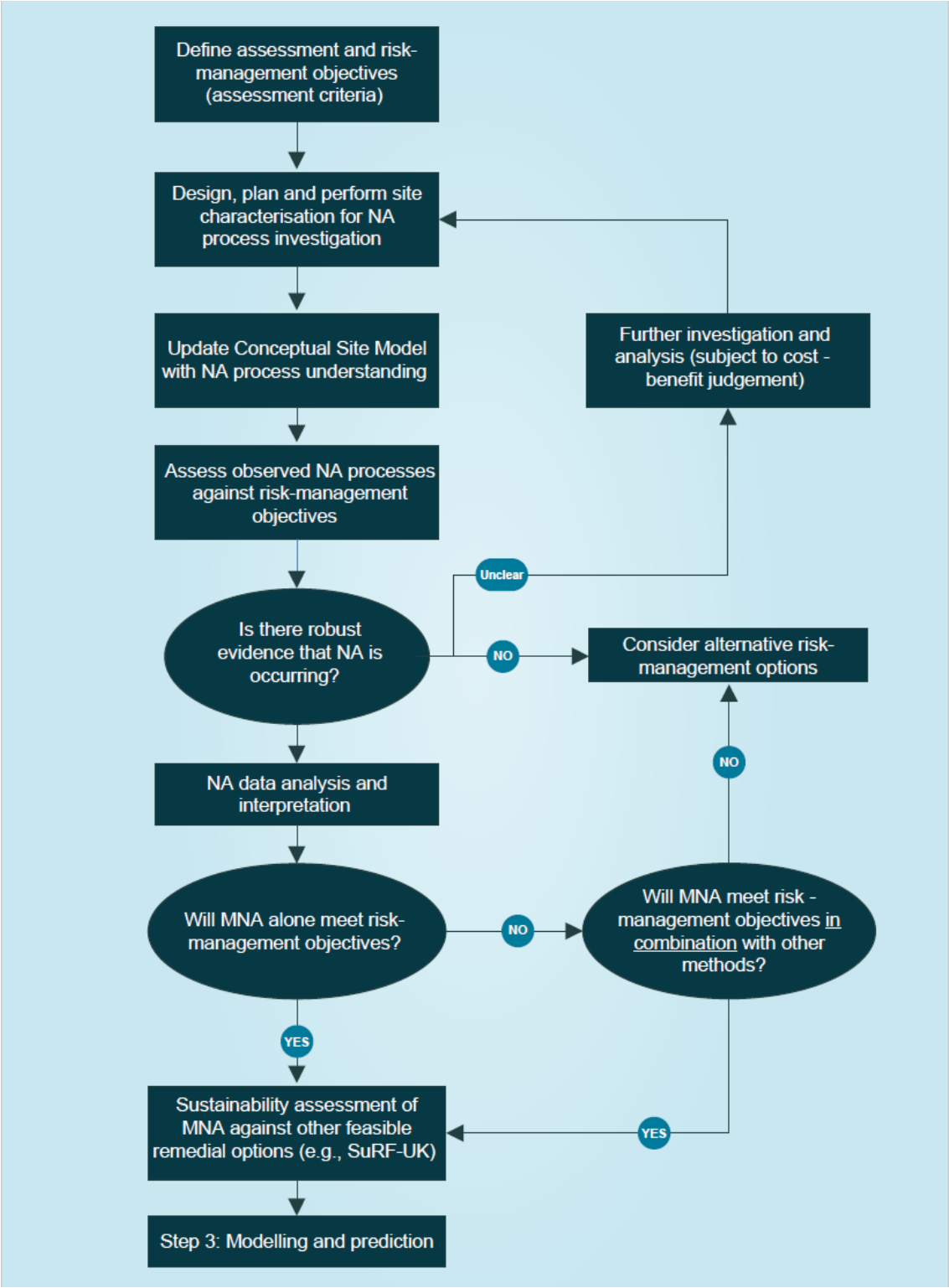


Figure 3: Step 2 – field demonstration of natural attenuation.

Step 3: Prediction and Modelling Future Natural Attenuation Behaviour

If the assessment completed at Step 2 shows that NA processes are currently occurring at a rate that manages any potential risks, Step 3 follows and requires an assessor to consider how the CoPC will behave in the future under a range of reasonable and foreseeable scenarios.

Trends in groundwater quality (and supporting lines of evidence) are extrapolated into the future so that assessors can predict the future performance of MNA, and can estimate the duration over which NA processes might need to be relied on to reach project closure.

The future plume predictions should consider the medium to long-term system and how changes might affect the success of an MNA solution. It is important that any remediation project is resilient to changing circumstances such as:

- significant water level, flow regime and water chemistry changes (e.g. as a result of climate change, or flood events);
- foreseeable changes to land use that may change existing or introduce new source-pathway-receptor linkages, or which restrict access to monitoring infrastructure; and
- foreseeable changes to land ownership that may make long-term access or accountability for remediation difficult.

Hydrogeological models, including simple tools such as Remedial Targets Worksheet (RTM) and ConSim, and NA-process models such as CoronaScreen, BioScreen, BioBalance and BIOCHLOR can be used to predict future concentration trends and whether MNA remains effective for a range of potential future scenarios. Where more complex hydrogeological simulation is required, numerical reactive transport models may provide greater insight (Appendix 7 – models).

The outcome of Step 3 is confirmation (or otherwise) that NA processes can be relied on to act in the future, and lead to achievement of remediation objectives. Given the potentially extended timescale for NA processes to reach remedial target concentrations, wider risk management requirements (i.e. institutional controls to prevent creation of new exposures, or long-term access rights to monitoring infrastructure) may be helpful.

Step 4: Implementation – Performance Monitoring and Verification of Monitored Natural Attenuation Projects

If an MNA strategy is agreed by the relevant parties at Step 3, the project progresses to the implementation phase (Appendix 6). Regular groundwater monitoring is undertaken to collect representative data to confirm MNA effectiveness. The frequency of monitoring should take account of:

- the range of hydrogeological conditions (e.g. seasonal variation);
- groundwater / CoPC transport velocities (i.e. sample rapidly-moving groundwater more frequently than slow-flowing);
- distance and travel-time to receptors; and
- provide ability to initiate an alternative course of action in a timely manner if MNA proves ineffective.

Monitoring is likely to be more frequent in Step 2, reducing in frequency in Step 4 and ultimately ceasing once trends are established and it has been demonstrated that risks are being managed.

The duration of monitoring is likely to reflect the potential risks present at site if MNA is not effective, and also the level of confidence in attenuation of the CoPC (Figure 2). Monitoring for NA of a moderate concentration petrol (BTEX) plume (which is readily biodegradable in aerobic conditions) may only require a few years data; whereas a plume of tetrachloroethene (PCE) from a DNAPL source in a deep aquifer (biodegradable under anoxic conditions) may require a greater, and perhaps much greater, duration of monitoring data to confidently demonstrate trends in concentration, plume stability and footprint towards remedial goals.

As performance monitoring data are collected, assessors will need to review the data and confirm that the strategy continues to manage potential risks. If it fails to manage the identified risk adequately, or new and unacceptable risks are identified, an alternative strategy may need to be put in place. A Contingency Plan should be developed that identifies alternative approaches if MNA is unsuccessful.

Ultimately, if MNA is successful, a verification report will document the effectiveness of the works (Environment Agency, 2023). The Environment Agency's guidance on verification follows a very similar lines of evidence approach to MNA set out in this document. Reporting project verification should draw on the existing CSM, NA lines of evidence, and performance monitoring data to confirm whether MNA has adequately mitigated risks, and further monitoring is therefore unnecessary.

A step-wise approach to implementation, monitoring and verification is provided in Figure 4.

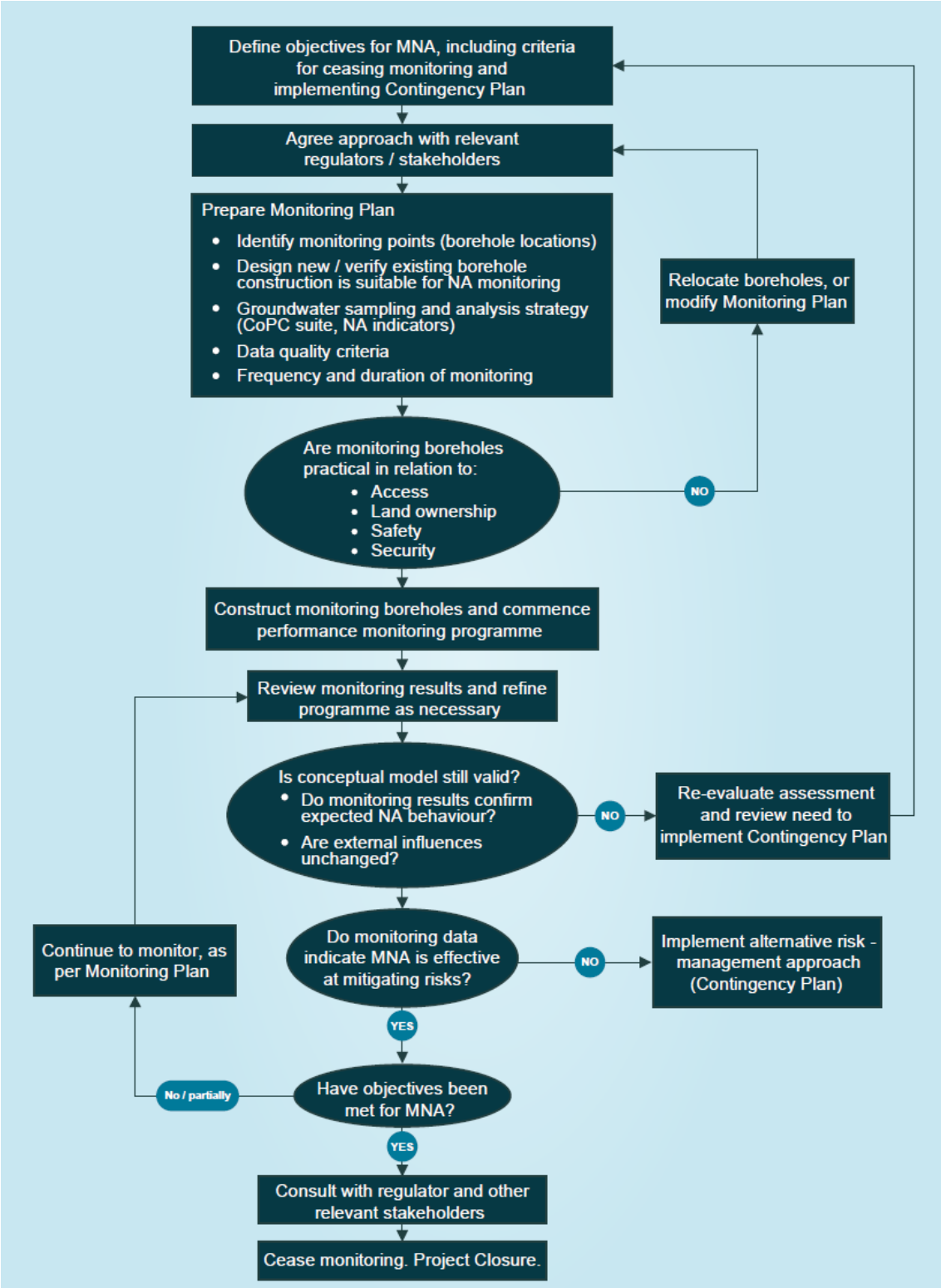


Figure 4: Step 4 – implementation, monitoring and verification.

Appendix 1: Summary of Screening Criteria to Assess the Feasibility of Monitored Natural Attenuation

Table A1.1: Summary of screening criteria for assessing the feasibility of MNA.

Screening criteria	Feasibility of MNA Strategy		
	High	**Moderate**	**Low**
A. Technical factors			
Primary source of groundwater contamination[1]	Release stopped. Soil and groundwater impact removed or being removed	Release stopped. Impact not removed	***Release continuing. Input to groundwater continuing***
Plume delineation	Fully delineated	Partially delineated, including in direction towards receptors	Poorly delineated
Contaminant plume (dissolved in groundwater) status	Shrinking	Stable	Expanding
Non-aqueous phase liquid (NAPL) presence	Absent	Residual saturation, or stable/shrinking NAPL footprint	Mobile NAPL, and expanding NAPL footprint
Persistence of CoPC in groundwater	Readily attenuated (degraded) under conditions present on site	Not readily degraded under conditions present on site	Attenuation processes poorly understood
Dominant attenuating mechanisms	Irreversible and destructive		Reversible and non-destructive
Mobility of CoPC[2]	Medium	Low	High
Pollution potential[3] of daughter products	Less polluting than parent compound	Equally polluting	More polluting than parent compound
Combined effects of multiple contaminants	No effect - attenuation occurs independently	Act as co-contaminants	Impose inhibitory effects
Aquifer heterogeneity	Homogeneous	Moderate heterogeneity (e.g. layered porous media)	Highly heterogeneous (e.g. highly fractured / karst)
Rate of groundwater migration	Slow	Medium	Rapid
Receptor	No receptors (e.g. abstraction wells, surface water) identified	Receptors present (low risk)	***Receptors present (high / imminent risk)***
Groundwater Source Protection Zones (SPZ)	Lies outside SPZ	Lies within SPZ III	***Lies in SPZ I*** or SPZ II[4]
Current and foreseeable groundwater use[5]	Low	Medium	High

Screening criteria	Feasibility of MNA Strategy		
	High	**Moderate**	**Low**
Level of confidence in monitoring data	High - comprehensive monitoring dataset spanning multiple seasons and years	Moderate - comprehensive monitoring dataset spanning multiple seasons	Low - single set of monitoring data
Confidence and understanding of contaminant distribution	High (e.g. dissolved substances in shallow homogeneous aquifer)		Low (e.g. DNAPLs in deep heterogeneous aquifer)
B. Regulatory factors			
Acceptability to the regulator	No policy objection No authorisation required	No policy objection Authorisation required	Policy objections in principle Authorisation refused
C. Sustainability, practicability and economic factors			
Monitoring locations	Access confirmed for on-site and off-site monitoring in the long term	Access possible for on-site and off-site monitoring in the long term	Limited / no access
Financial provisions	Long-term, legally-binding budget secured	Long-term, non-legally binding budget secured	No long-term budget in place
Objectives of landowner	Long-term interest in site (>10 years)	Medium-term interest (3 - 10 years)	Short-term ownership/ developer (<3 years)
Sustainability	More sustainable than alternative options		Less sustainable than alternative options
OVERALL	All high / intermediates No lows	High, medium and lows, but no show-stoppers*	One or more show-stopping criteria present, or No factors of high feasibility rating

* Criteria highlighted in bold italics would normally preclude MNA as a sole remedial option

1 Primary source of contaminants to groundwater, e.g. leaking pipe, sewer, tank, leachable/mobile contaminants in the deposited materials/soil/unsaturated zone.
2 Medium mobility enables degradation products to be removed, thus driving degradation reactions.
3 Pollution potential is a function of the persistence, mobility and toxicity of the contaminant.
4 Source Protection Zones defined in England Wales. For SPZ II which have been defined by the Environment Agency using the 25% of total of SPZ III (i.e. low groundwater flow velocity aquifers), then site-specific factors may increase the feasibility of NA.
5 Groundwater uses that should be considered include: water abstraction (e.g. public water supply), baseflow to surface waters and groundwater-dependent terrestrial ecosystems.

Appendix 2: Processes Involved in Natural Attenuation

A2.1 Introduction

Natural attenuation (NA) is the reduction of CoPC concentrations in the environment through three main processes:

1. Physical phenomena (advection, dispersion, diffusion, matrix diffusion, dilution and volatilisation);
2. Geochemical reactions (sorption and chemical or abiotic reactions); and,
3. Biochemical processes (aerobic and anaerobic biodegradation).

Some of these processes may simply redistribute contaminant mass within the mobile phase (e.g. dispersion), some transfer the contaminant from the mobile phase to an immobile phase (e.g. sorption, which results in retardation) and some result in a loss in contaminant mass (i.e. are destructive, such as degradation).

This section will provide an overview of the processes of NA for common environmental contaminants such hydrocarbons and chlorinated ethenes. Equations for calculating the rate of contaminant degradation for MNA are also provided.

A summary of the main processes affecting contaminant transport is provided in Table A2.1, with further details provided throughout the section.

Table A2.1: Summary of important processes affecting solute fate and transport (modified from Wiedemeier *et al*. 1999).

Category	Process	Description	Dependencies	Effect and implications for MNA
Physical processes	Advection	Movement of solute by bulk groundwater movement.	Dependent on aquifer properties, mainly hydraulic conductivity and effective porosity, and hydraulic gradient. Independent of contaminant properties.	Main mechanism driving contaminant movement in the subsurface and is typically calculated and presented as average linear groundwater velocity, also termed seepage velocity. It does not result in a loss of contaminant mass.
	Dispersion	Mixing of fluid and solutes due to groundwater movement and aquifer physical heterogeneities	Dependent on aquifer properties (e.g. variation in pore size and geometry, layering etc.) and scale of observation. Independent of contaminant properties.	Causes longitudinal, lateral, and vertical spreading of the contaminant plume. Reduces solute concentration but does not result in mass loss.
	Diffusion	Spreading and dilution of contaminant due to molecular diffusion.	Dependent on contaminant and aquifer properties such as grain size variation and contaminant concentration gradients. Described by Fick's Laws.	Diffusion of contaminant from areas of high concentration to areas of low concentration. Generally unimportant relative to dispersion, except for very fine-grained porous media where advection is very low – in which case molecular diffusion can be an important component of hydrodynamic dispersion. Does not result in loss of contaminant mass.

Category	Process	Description	Dependencies	Effect and implications for MNA
Physical processes (cont.)	Matrix diffusion	Diffusion into a low permeability zone within an aquifer of heterogeneous permeability.	As above within an aquifer of heterogeneous permeability, for example, bands of silt in a sand and gravel aquifer. In fractured dual porosity formations such as a Chalk aquifer, matrix diffusion is important for solute transport and NA.	A two-step process; (1) Contaminant diffusion occurs relatively slowly within low permeability bands in an aquifer, temporarily sequestering a proportion of the contamination (loading); (2) Following a reduction in the concentration of contamination in higher permeability zones, the slow diffusion of contamination out of low permeability zones results in a gradual contaminant release into the higher permeability aquifer over an extended timeframe, extending the lifetime of plumes ("back-diffusion"). In fractured dual porosity aquifers, contaminants diffuse from the fracture pore water into the matrix pore water during loading/plume migration, and diffuse back into the fracture water when contaminant loadings decrease. See Thornton *et al*. (2006).
	Recharge	Movement of water into the saturated zone.	Dependent on aquifer matrix properties, depth to groundwater, depth to contaminant plume, surface water interactions, and climate	Causes dilution of the contaminant plume and may replenish electron acceptor concentrations, especially dissolved oxygen.

Category	Process	Description	Dependencies	Effect and implications for MNA
Physical processes (cont.)	Volatilisation	Volatilisation of contaminants dissolved in groundwater into the vapour phase (soil gas).	Only occurs at air-water interface.	Removes contaminants from NAPL (described by Vp) and groundwater (described by H) and transfers them to soil vapour. This is typically more significant in shallow water tables. Relative to biodegradation, this is normally a minor component of MNA. Further reading is provided in Technical Bulletin 20 (CL:AIRE, 2019a) and emergent NSZD good-practice guidance from CL:AIRE (2024).
Geochemical processes	Sorption	Reversible partitioning between aquifer matrix and solute whereby contaminants become sorbed onto solid phase, principally organic carbon and clay minerals, or metal oxides / hydroxides.	Dependent on aquifer matrix properties (organic carbon, clay and mineral content, bulk density, specific surface area, and porosity) and contaminant properties (solubility, hydrophobicity, octanol-water partitioning coefficient for organic contaminants).	Tends to reduce apparent solute transport velocity or can remove solutes permanently from the groundwater via sorption to the aquifer matrix, however, it is not considered that solutes are permanently removed as desorption may occur. Sorption does not result in a net loss of contaminant mass.
	Abiotic degradation	Chemical transformations that degrade contaminants without microbial facilitation, such as hydrolysis.	Dependent on contaminant properties, aquifer and groundwater geochemistry.	Can result in partial or complete degradation of contaminants. Rates of overall mass destruction are typically slower than for biodegradation. Results in a loss of contaminant mass.

Category	Process	Description	Dependencies	Effect and implications for MNA
Geochemical processes (cont.)	Partitioning from NAPL	Partitioning from NAPL into groundwater. NAPL, whether mobile or residual, tend to act as a continuing source of groundwater contamination.	Dependent on aquifer matrix and contaminant properties (such as NAPL composition and effective solubility of organic compounds according to Raoult's Law), as well as groundwater mass flux through or past NAPL.	Dissolution of contaminants from NAPL represents the primary source of dissolved contamination in groundwater. It should be noted that the composition of the dissolved phase plume will vary over time with ongoing NAPL dissolution, as approximated by the temporal variation in effective solubility of the NAPL constituents. This can influence the monitoring priorities over the project duration.
Biochemical processes	Biodegradation	Microbially mediated oxidation-reduction reactions that degrade contaminants.	Dependent on groundwater geochemistry and aquifer geochemical properties, microbial population and contaminant properties. Biodegradation can occur under aerobic and/or anaerobic conditions.	May ultimately result in complete degradation of contaminants. Typically, the most important process acting to reduce contaminant mass. It should be noted, however, that biodegradation does not always result in mineralisation. Metabolic intermediates of contaminants can form, such as cis-1,2-dichloroethene from the biodegradation of trichloroethene.

Figure A2.1 illustrates the different concentration profiles that would be expected for advection, dispersion, sorption and degradation.

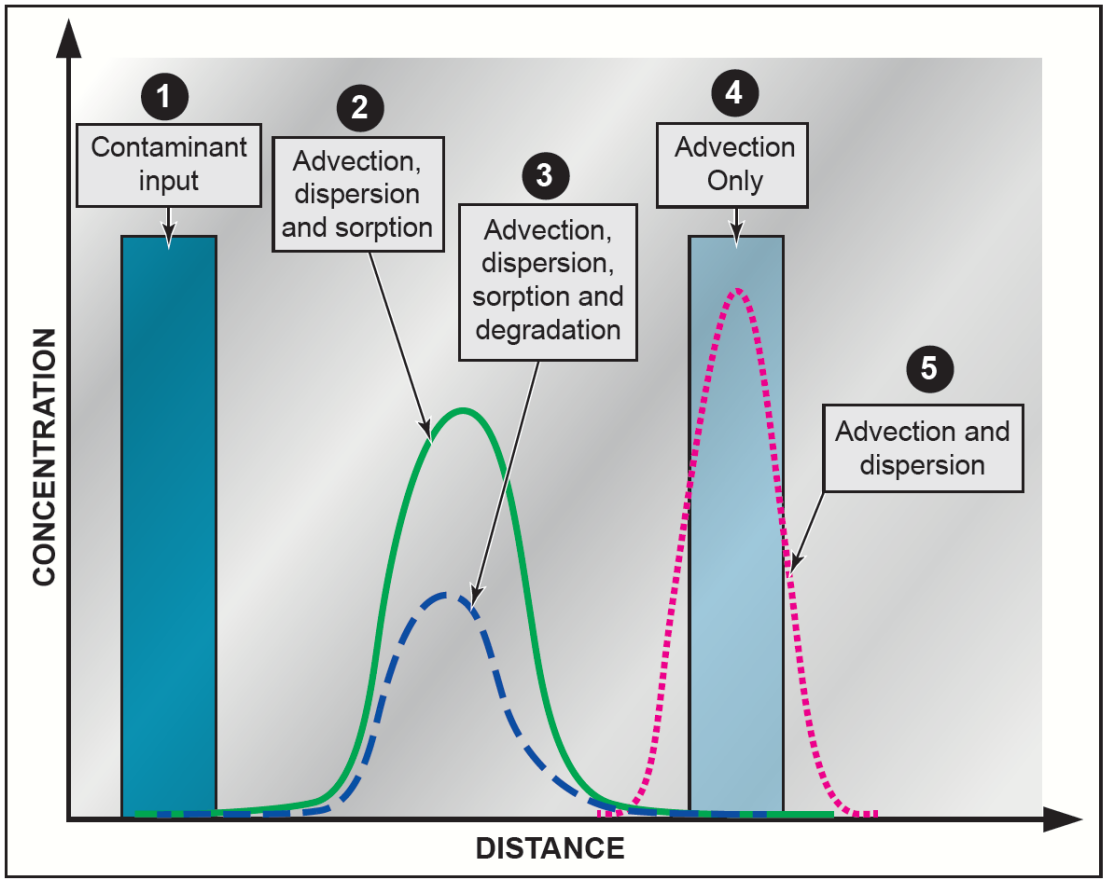


Figure A2.1: Idealised section along a contaminant flow path to illustrate influence of advection, dispersion, sorption and degradation, after a given duration of time.

The concentration of a contaminant introduced into an aquifer is shown as Point 1 in Figure A2.1. At Point 2, processes such as sorption together with the gradual release of the contaminant from the aquifer matrix result in a lower concentration with distance from the source, but with greater spreading of the contaminant peak. Point 3 shows the same active processes as in Point 2, but with degradation occurring simultaneously. This results in a lower curve peak due to destructive loss of contaminant mass. If advection occurs alone, the spread of the contaminant peak is limited by the effective solubility of the contaminating substance, and can mirror the initial contaminant input concentration if sufficiently water soluble (Point 4). With dispersion and advection together (Point 5), the solute concentration is reduced due to longitudinal, lateral, and vertical spreading of the contaminant peak.

A2.2 Physical Processes

Advection is the main process in the migration of contaminants and is driven by the properties of the media, independent of the molecular physical or chemical properties of the contaminant. It describes the transport of dissolved substances (solutes) by groundwater under a hydraulic gradient. Non-reactive (conservative) substances travel

at the same rate as water. Reactive solvents may be retarded by other processes and travel more slowly than the groundwater. The equation for one-dimensional advective transport is given in Guerrero and Skaggs (2010).

Dilution is a physical process of NA that reduces the concentration of a contaminant, but does not affect its total mass, toxicity or mobility. It describes the mixing of contaminated water by clean groundwater. It is likely to be an important mechanism in reducing concentrations, wherever small quantities of contaminant reach the aquifer with a comparatively large groundwater flow or throughput. Further dilution can occur by:

- Uncontaminated infiltration (recharge of precipitation and infiltration of surface waters (lakes, rivers)) away from the source area (this is the only mechanism of dilution that is strictly applicable to NA);
- Contaminated groundwater discharging to a clean surface water body or mixing with clean water at an abstraction point.

Infiltration can be an important mechanism in introducing electron acceptors (dissolved oxygen, nitrate, sulfate) where contaminants are being biodegraded.

Dispersion will reduce contaminant concentrations by spreading the contaminant (in a longitudinal, lateral and vertical direction) as groundwater flows through the aquifer. It reduces the concentration of a contaminant, but does not affect its total mass, toxicity or mobility. Dispersion can facilitate biodegradation by reducing contaminant concentrations below toxic thresholds and spreading the plume into areas with electron acceptors. Further information about dispersion and biodegradation can be found in Wilson *et al*. (2005).

Dispersion occurs due to mechanical dispersion and molecular diffusion and can be represented by the following equations:

$$D = D^d + D^* \qquad \text{Equation A2.1}$$

and

$$D = \propto D_w + \alpha.v \qquad \text{Equation A2.2}$$

where

D = hydrodynamic dispersion (m^2/s)

D^* = mechanical dispersion (m^2/s)

D^d = molecular diffusion coefficient through medium (m^2/s)

α = dispersivity (m)

v = groundwater velocity (m/s)

$\propto$ = tortuosity of medium

D_w = molecular diffusion coefficient in water (m^2/s)

For many groundwater systems, diffusion is small or negligible compared to mechanical dispersion and Equation A2.2 reduces to $D = \alpha.v$

Mechanical dispersion is the main process in spreading contaminants and is a result of variation in the velocity of water movement through pores of different size, tortuosity (flow path length), and frictional variations within the pore space. Dispersion has a longitudinal (parallel to the flow direction), transverse (perpendicular to the flow direction) and vertical component. As the scale of the plume or system increases, dispersion will also increase (i.e. it is scale dependent). The value of dispersion will directly reflect the heterogeneity of the system. Further formulas and a more detailed description can be found in Freeze and Cherry (1979).

Diffusion is observed as the movement of contaminants from regions of higher concentration to lower concentration but occurs due to random atomic scale movement of atoms and molecules. It reduces the concentration of a contaminant, but does not affect its total mass, toxicity or mobility. Diffusion is slow in comparison to mechanical dispersion, and only becomes significant in no-flow or very low-flow systems, or over very long timescales. Diffusion is a key process in bringing electron acceptors and electron donors together with bacteria and transferring solutes to surfaces. This is because diffusion is highly significant in vertical transport and dispersion processes and to a lesser extent in horizontal transport and dispersion, thereby allowing lateral mixing into plumes.

Matrix diffusion occurs in aquifers with variable high and low permeability bands such as sands and gravels containing silt layers. Diffusion of contamination into a low permeability zone, temporarily sequesters contamination. Following a reduction in the concentration of contaminants within the high permeability zones, a concentration gradient is formed, and diffusion slowly occurs out of the low permeability zone back into the aquifer, typically extending the lifetime of plumes. The process, is shown in Figure A2.2, with the consequence of ongoing or renewed contamination of groundwater commonly referred to as “rebound”.

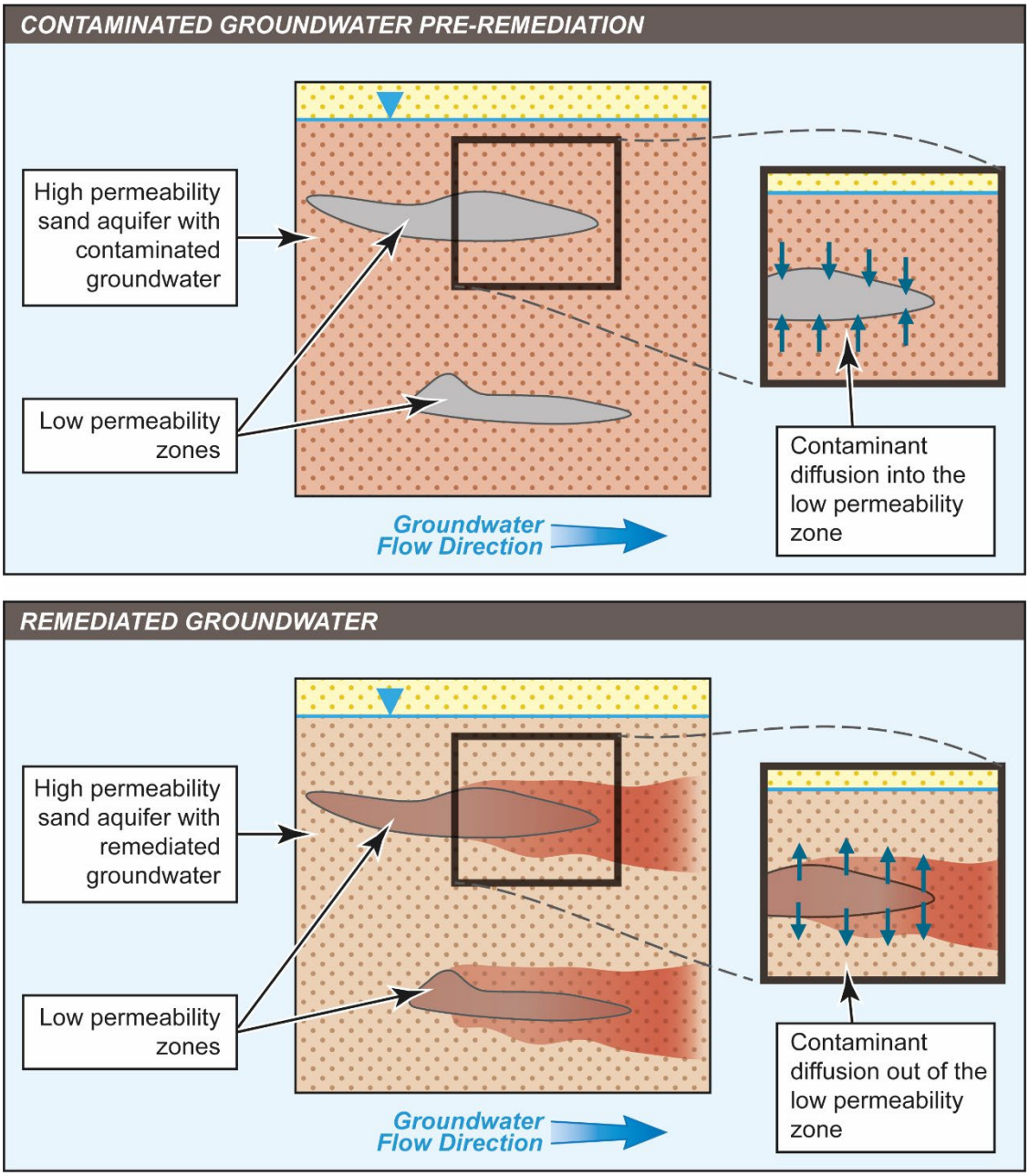


Figure A2.2: Matrix diffusion (Source: WSP).

For dual porosity systems, such as fractured sandstone aquifers and the Chalk, diffusion of contaminants from the mobile fissure water to the less mobile pore water can be an important mechanism in retarding contaminant movement, and is referred to as rock matrix diffusion.

Below is an equation describing the diffusive flux in porous media (based on Equation 3 and Equation 8 in Parker *et al*., 1994).

$$J_D\ (0, \mathrm{t}) = \Phi C_s \sqrt{\frac{RD_e}{\pi t}}$$ Equation A2.3

$$\frac{D_e}{D_o} \equiv \tau \approx \Phi^P$$ Equation A2.4

Equation A2.3, where:

J_D = diffusive flux at time t [M/T/L^2] where M is mass, L is length and T is time

Φ = porosity [L^3/L^3]

C_s = solute concentration [M/L^3] (often taken as pure phase solubility with symbol S_w [M/L^3])

R = retardation factor [-]

D_e = effective diffusion coefficient [L^2/T]

t = time [T]

Equation A2.4, where:

D_e = effective diffusion coefficient [L^2/T]

D_0 = diffusion coefficient in water [L^2/T]

τ = tortuosity

Φ = porosity [L^3/L^3]

P = an exponent factor with values between 1.3 and 5.4 depending on the geologic material

Volatilisation of volatile contaminants to soil vapour occurs at the capillary fringe and results in removal of contaminant mass from the groundwater, but is not inherently destructive. Volatilisation is dependent on the physico-chemical characteristics of the contaminant, and is dependent on site-specific conditions including temperature, depth to water and porosity. This is generally not a significant mechanism due to the area of contaminated groundwater exposed to soil gas. Also, as the capillary fringe is quasi-immobile, transfer across it is dominated by aqueous phase diffusion coefficients, which are around four orders of magnitude lower for volatile organic compounds (VOCs) in the liquid phase, than in the gas phase. The limited vertical dispersion across the capillary fringe is dominated by diffusion, therefore VOCs struggle to transfer. However, once at the capillary fringe, partitioning of a volatile substance from the dissolved phase into the vapour phase is described by its Henry's Law constant:

$$C_V = H \times C$$

Where*:*

C_V = concentration in vapour phase (mg/l)

H = Henry's Law Constant (dimensionless)

C = concentration in aqueous phase (mg/l)

A2.3 Geochemical Processes

Sorption describes the interaction of a contaminant between water and soil. This process will reduce contaminant concentrations by their removal from solution due to interaction with the matrix of the aquifer through which groundwater is moving. There is no mass reduction of the contaminant. Sorption can occur as a result of:

- adsorption, the attachment of a solute to a soil particle's surface;
- absorption, the movement of a solute (diffusion) into the structure of a porous particle where it sorbs onto an internal surface; and
- ion exchange, the replacement of a sorbed ion by the contaminant.

Sorption will retard the rate at which contaminants move through the system. The retardation of a contaminant can be defined as:

$$Retardation\ (Rf) = \frac{v}{u}$$ Equation A2.6

Where:

Rf = retardation factor

u = velocity of contaminant or solute (m/d)

v = velocity of groundwater flow (m/d)

Further information and calculations for retardation can be found in Lovanh *et al*. (2000).

Sorption and desorption kinetics refer to the rate at which a contaminant either attaches to or detaches from a sorbent. Desorption is generally slower than sorption, such that contaminant concentrations are reduced, although the sorbed contaminant can represent a longer-lasting source than those dissolved within groundwater.

Sorption is a function of:

- the nature of the contaminant (conservative contaminants such as chloride are not sorbed, whereas reactive contaminants, such as metals can be strongly sorbed);
- the contaminant (solute) concentration;
- the nature and concentration of other contaminants (competition with other contaminants can reduce the number of sites for sorption or competition with other cations);
- nature of the soil/rock matrix, including surface area;
- presence of clay, organics and oxyhydroxides which can provide sites for sorption;
- environment, the pH and redox potential of the system can influence sorption. The sorption of some metals is very sensitive to pH and redox conditions; and
- flow rate, in terms of the kinetics of sorption.

For non-polar organic and inorganic contaminants sorption occurs preferentially to soil organic matter or to clay minerals, and sorption of metals occurs to oxides and hydroxides. In most aquifers, sorption to organic matter is the dominant process, except where the organic content is low and then sorption to mineral surfaces is the main process (Ball and Roberts, 1991).

When considering sorption to organic matter as a general process, it is important to distinguish between K_{oc} (organic carbon-water partitioning coefficient) and K_{om} (partitioning coefficient normalised to soil organic matter). The use of these terms will depend on what is measured in the aquifer material. It is possible to convert K_{om} to K_{oc} using a conversion factor of 1.724 (K_{oc} = 1.724 K_{om}) (Dragun, 1988).

In situations where thermally altered carbonaceous material (TACM) is present it may produce anomalously high K_{oc} values due to the enhanced sorption to TACM. This could lead to wrong estimates of sorption by order of magnitude. The issue is described in more detail in Wang *et al.* (2013) and Rivett *et al.* (2019).

The partition coefficient for the sorption of organic contaminants to organic matter can be calculated as follows:

Partition coefficient for non-polar organic chemicals (e.g. aromatic hydrocarbons such as benzene, toluene):

$$K_d = K_{oc} \times f_{oc} \qquad \text{Equation A2.7}$$

Partition coefficient for ionic organic chemicals (e.g. phenol)

$$K_d = K_{oc,n} (1 + 10^{(pH-pKa)})^{-1} + K_{oc,i} (1 - (1 + 10^{(pH-pKa)})^{-1}) \qquad \text{Equation A2.8}$$

Where:

- K_d = soil-water partition coefficient (l/kg)
- K_{oc} = organic carbon partition coefficient (l/kg)
- f_{oc} = fraction of organic carbon (fraction)
- $K_{oc,n}$ = sorption coefficient for related species (l/kg)
- $K_{oc,i}$ = sorption coefficient for ionised species (l/kg)
- pH = pH value
- pKa = acid dissociation constant

The partition coefficient (K_d) describes the distribution of a solute between groundwater and the solid and is typically represented by either:

1. Linear isotherm

$$K_d = \frac{C}{C_s}$$ Equation A2.9

2. **Freundlich isotherm**

$$K_d = \frac{C^{\frac{1}{N}}}{C_s}$$ Equation A2.10

3. Langmuir isotherm

$$K_d = \frac{C_s}{C(b - C_s)}$$ Equation A2.11

Where:

K_d = partition coefficient (l/kg)

C = concentration in the aqueous phase (mg/l)

Cs = concentration in the solid phase (mg/kg)

b = maximum amount of contaminant that can be sorbed (g/g)

N = chemical-specific coefficient (values of 1/N typically range from 0.7 to 1.1)

Solutes sorbed onto colloids (colloidal sorption) may be transported through the aquifer system. Colloidal particles of sub-micron sized organic matter and minerals occur naturally in soils and groundwater, and have been found to play a role in the transport of trace metals and radionuclides (Honeyman, 1999).

Complexation. Metal ions in aqueous solution are typically present as complexes. A complex is an ion in a combination of cations with anions or molecules. Chelating agents such as humic substances which may be found in landfill leachate can form soluble complexes with heavy metals such as nickel and zinc which can be highly mobile in the environment. Soils and aquifer materials components differ greatly in their sorption capacities, their cation and anion exchange capacities, and the binding energies of their sorption sites. Polyvalent cations (e.g. Zn, Cu, Ni) may be strongly adsorbed on phyllosilicates (e.g. clay minerals) due to the presence of –SiOH or –AlOH groups capable of chemisorbing these ions. Organic matter and variable charge minerals (Mn, Fe and Al oxides) are much more effective scavengers of polyvalent cations because complexation processes are the dominant binding mechanisms. Anionic forms of elements sorb primarily to variable charge minerals, carbonate, and at the edges of phyllosilicates. They are not typically sorbed on soil organic matter, but certain elements (e.g. borate, arsenate, arsenite, selenite) can bind to humic substances. As the variable charge is pH-dependent and varies with pH, anionic sorption to variably charged surfaces will increase with pH.

Oxidation/reduction. A chemical or biological reaction where an electron is transferred from an electron donor to an electron acceptor and results in a change in the valence state of the ion. In many cases the solubility of the ion will also be different, giving rise to precipitation or sorption of the ion. For example, hexavalent chromium (soluble) occurs under oxidising conditions. If conditions become reducing, this is converted to trivalent chromium (insoluble) and this metal is precipitated out of solution. Changes to redox conditions may reverse these reactions.

Solution/precipitation. Contaminants may be precipitated out of solution if physiochemical conditions change. For example, changes to pH and water chemistry (e.g. ionic strength and ionic composition) can cause dissolved metals to precipitate out of solution or become dissolved. This includes (i) precipitation of metal oxyhydroxides and carbonates under alkaline pH conditions or in the presence of carbonate ions and (ii) precipitation of metal sulfides under anaerobic sulfate-reducing conditions in the presence of sulfide ions.

A2.4 Chemical or Abiotic Degradation

Biodegradation is often considered the dominant destructive NA mechanism in groundwater. However, several common groundwater contaminants can also degrade through abiotic processes, that, in some cases, may be the primary or only destructive process occurring (Brown *et al*., 2007). Abiotic chemical degradation occurs when a compound reacts in natural conditions without catalysis by microbes or other life forms (Adamson and Newell, 2014). This section focuses on abiotic degradation processes for chlorinated solvents, as these are common groundwater contaminants and owing to the complexity of the reactions potentially occurring. The detoxification or degradation by abiotic processes of other groundwater contaminants, such as MTBE, chromium (VI) and uranium (VI), are presented elsewhere in the literature (e.g. Elsner *et al*., 2007; Hyun *et al.*, 2012; Lee and Batchelor, 2002).

Since the 1970s, it was understood that trichloroethanes and tetrachloroethanes underwent spontaneous abiotic degradation in groundwater via hydrolysis to form 1,1-dichloroethene (1,1-DCE) and trichloroethane respectively (Mabey and Mill, 1978; Jeffers *et al.,* 1989). Most other chlorinated solvents were assumed to be resistant to abiotic degradation, so these processes were largely overlooked in MNA protocols published during 1990s and early 2000s (e.g. Wiedemeier *et al.,* 1998; Environment Agency*,* 2000). However, understanding of abiotic degradation reactions, particularly those associated with catalytic reactions on surfaces of iron-rich minerals (Brown *et al*., 2007; He *et al.,* 2009; He *et al*., 2015), has been advanced over the past 15 years, and approaches to quantify the contributions of iron-bearing minerals to contaminant degradation are now available (He *et al*., 2009; Lebrón *et al*., 2015; Wiedemeier *et al*., 2017).

Initial research focused on carbon tetrachloride, 1,1,1-trichloroethane (1,1,1-TCA), tetrachloroethene (TCE) and tetrachloroethene (PCE) abiotic degradation processes on mineral surfaces including iron sulfides (FeS, pyrite), magnetite and so-called ‘Green Rusts’, that can occur naturally in subsurface anaerobic environments. The significance of iron-bearing minerals to chlorinated ethene NA is such that the abundances of magnetite (indicated by magnetic susceptibility measurements) and FeS (iron sulfide) are amongst five key parameters correlated with TCE, cis-1,2-dichloroethene (cis-DCE)

and vinyl chloride (VC) degradation rates in recent industry research (Lebrón *et al*., 2015).

It is important to note that these reactive iron minerals are often biogenically formed. For example, iron-reducing and/or sulfate-reducing bacteria may be responsible for the formation of iron sulfide, which is involved in the reductive dechlorination of a contaminant. Consequently, these reactions are often referred to as biogeochemical, or biologically-mediated abiotic degradation (BMAD), to acknowledge the biological component (Adamson and Newell, 2014).

Abiotic degradation is typically more favourable for the more chlorinated compounds (trichloro-, tetrachloro- etc) compared to the less chlorinated compounds ([mono]chloro-, dichloro-). In general, rates of abiotic degradation are slower than biotic degradation rates. However, abiotic processes may be important for NA of chlorinated solvents where high mass loadings of reactive minerals are generated *in situ* or where the activity of dechlorinating bacteria is low.

A2.5 Biochemical Processes

A2.5.1 Biodegradation

Biodegradation is the main process in the NA of organic contaminants and results in a mass loss (destructive) and is typically estimated using a first-order decay model, although other conceptual models (e.g. instantaneous reaction) can be used to describe biodegradation processes, if data allows, as emphasised in the suite of numerical models described in later appendices. Organic compounds are biodegraded via either oxidation or reduction of the organic contaminant, when electron donors, electron acceptors and nutrients are combined by microorganisms to produce metabolic by-products and energy for microbial growth. This can be represented by the following generalised equation.

Microorganisms + electron donor + electron acceptor + nutrients

↓

metabolic by-products + energy + microorganisms

Aliphatic and aromatic hydrocarbons (e.g. BTEX) serve as the electron donor and are broken down in the process. Electron acceptors, in order of preference for utilisation by microbes, include oxygen, nitrate, manganese (IV), iron (III), sulfate and carbon dioxide. Manganese and iron are typically present in the mineral form. Depending on the electron acceptor used, the metabolic by-products include carbon dioxide, water, nitrogen gas, manganese (II), iron (II), sulfide, dissolved hydrogen and methane. Specific organic intermediate compounds may also accumulate or be transiently detected with these reaction products during biodegradation. The intermediate compounds can be independent signatures of biodegradation, for example, TBA for ether oxygenate biodegradation.

Decreases in the concentration of soluble electron acceptors and corresponding increase in the concentration of metabolic by-products provide indirect evidence for degradation. Table A2.2 provides a summary of changes in contaminant, electron acceptor and metabolic by-product concentrations during biodegradation. The degradation process can vary in different parts of the plume, for example, anaerobic

degradation may be occurring at the centre of the plume and aerobic degradation at the margin of the plume.

Table A2.2: Trends in contaminant, electron acceptor and metabolic by-product concentrations during biodegradation (modified from Wiedemeier *et al.*, 1998).

Analyte	Trend in analyte concentrations during biodegradation	Terminal electron accepting processes causing trend[2, 3]
Petroleum hydrocarbons and polycyclic aromatic hydrocarbons (PAHs)	Decrease	Aerobic respiration, denitrification, Mn(IV) reduction, Fe(III) reduction, sulfate reduction, methanogenesis
Highly chlorinated solvents (3 or more Cl atoms) and daughter products	Parent compound concentrations decrease, daughter products increase initially and then may decrease	Reductive dechlorination and cometabolic oxidation
Lightly chlorinated solvents (2 or less Cl atoms)	Decrease	Aerobic respiration and Fe(III) reduction (direct oxidation) and cometabolism (indirect oxidation). Also reductive degradation to ethene, ethane.
Dissolved oxygen	Decrease	Aerobic respiration
Nitrate	Decrease	Denitrification
Mn(II)	Increase (metabolic by-product)	Mn(IV)[1] reduction
Fe(II)	Increase (metabolic by-product)	Fe(III)[1] reduction
Sulfate	Decrease	Sulfate reduction
Methane	Increase	Methanogenesis
Chloride	Increase	Reductive dechlorination or direct oxidation of chlorinated compound. In most cases, a significant difference is impossible to measure.
Redox (oxidation/reduction potential)	Decrease	Aerobic respiration, denitrification, Mn(IV) reduction, Fe(III) reduction, sulfate reduction, methanogenesis and halorespiration
Dissolved carbon dioxide	Increase	Aerobic respiration, denitrification, Fe(III) reduction and sulfate reduction

Notes:

1. *Mineral phase*
2. *Oxygen is the most favoured electron acceptor for microbes in the biodegradation of organics. Anaerobic bacteria cannot function if dissolved oxygen concentrations exceed 0.5 mg/l (i.e. if dissolved oxygen levels are greater than this aerobic degradation is the most likely process). Multiple processes can occur simultaneously within aquifers due to niche conditions in localised areas.*
3. *Microorganisms will generally use electron acceptors in the following order of preference: oxygen, nitrate, manganese, iron, sulfate, CO_2*

Figures A2.3 and A2.4 illustrate the geochemical evolution of a groundwater system contaminated with petroleum hydrocarbons. There are, however, two theories regarding the spatial distribution of electron acceptor use; (i) the redox zonation concept; and (ii) the plume fringe concept. These are shown in Figure A2.4. The redox zonation concept revolves around microorganisms preferentially and discretely utilising more thermodynamically-favourable electron acceptors. Recent literature, however, indicates that biodegradation within a plume of contamination may be better described by the plume fringe concept, in which the dissolved electron acceptors are depleted in the plume core, with biodegradation occurring by oxygen, nitrate or sulfate reduction at the fringes due to replenishment by surrounding groundwater (Meckenstock *et al.*, 2015; Thornton, 2019).

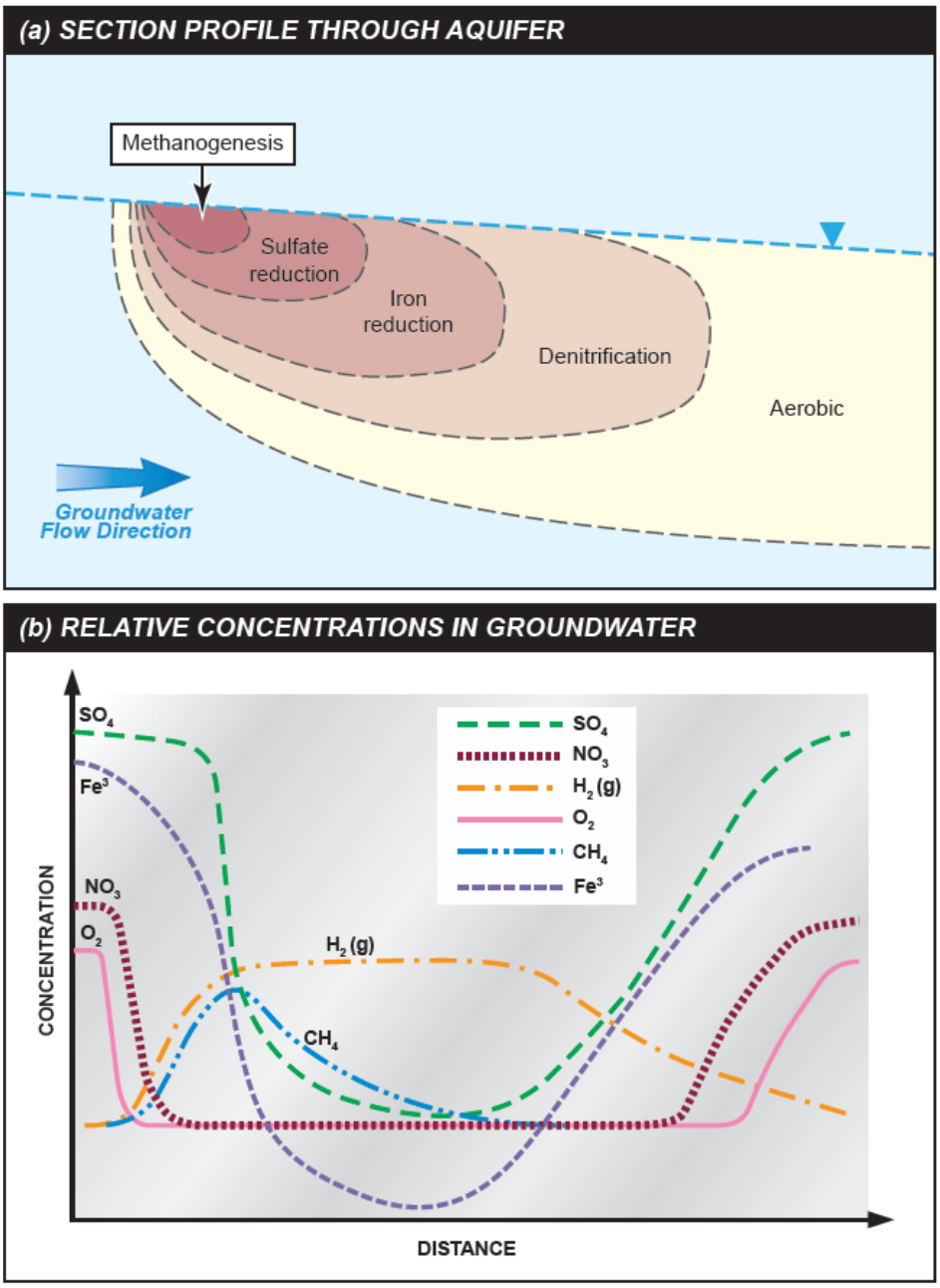


Figure A2.3: Conceptual section of (a) oxidation/reduction (redox) zones in groundwater, and (b) changes in distribution of electron acceptor and metabolic by-products in groundwater, with distance from contaminant source.

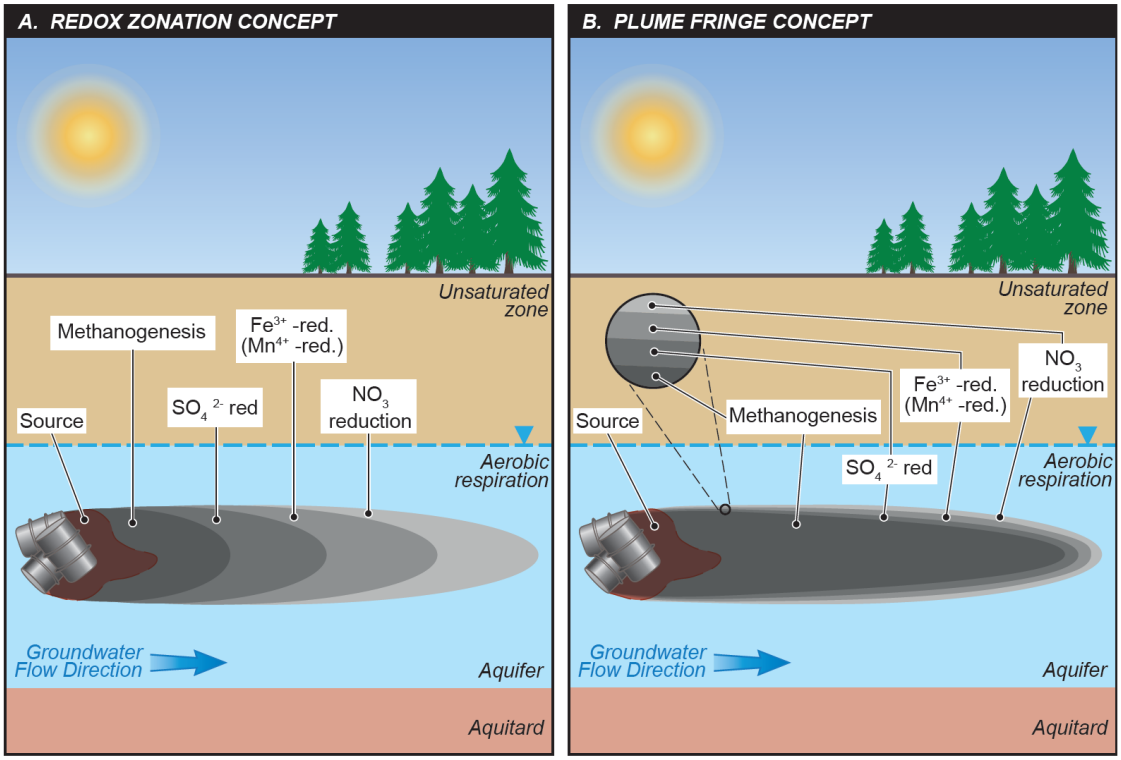


Figure A2.4: Comparison of redox zonation and plume fringe concepts within a hydrocarbon plume, both describing the spatial distribution of electron acceptors and processes of respiration. Reprinted (adapted) with permission from Meckenstock *et al*. (2015). © 2015 American Chemical Society.

The degradation of other organics can be more complex. Under anaerobic conditions, reductive dechlorination is the primary mechanism by which biotransformation of PCE and TCE occurs, and halorespiration (i.e. microorganisms capable of using chlorinated ethenes as terminal electron acceptors) is the process by which microorganisms dechlorinate chlorinated ethenes to ethene. This process is sequential PCE → TCE → cis-DCE → VC → ethene. The complete chlororespiration of cis-DCE and VC is known to occur by only a few species of *Dehalococcoides*, with the dechlorination of VC occurring most efficiently under highly reducing methanogenic conditions (Thornton *et al*, 2016). Complete dechlorination will only occur if sulfate is completely reduced, and a fermentable source of organic carbon is present to provide hydrogen as the electron donor (NICOLE, 2005; Xiao *et al.*, 2020).

Under the correct environmental conditions (noting that such conditions often require human intervention to be achieved and sustained), chlororespiration can play a significant part in the NA of chlorinated contamination, however, there are several potential causes of incomplete dechlorination, which frequently result in the accumulation of cis-DCE and VC (Bradley and Chapelle, 2010):

1. An insufficient supply of electron donors and nutrients/trace elements;
2. Competition for available hydrogen with other species of bacteria;
3. The presence of nitrate, which can act as an alternative electron acceptor;
4. Few or no microorganisms capable of dechlorinating cis-DCE and VC; and

5. Inhibitory substances such as chloroform or oxidised chlorinated ethene compounds present in the groundwater.

Inhibition of chlorinated ethene biodegradation can occur in areas in which high concentrations of sulfate are present due to sulfate-reducing bacteria out-competing dechlorinating microorganisms (such as *Dehalococcoides spp*.) for electron donors (such as hydrogen) and generating sulfide gas.

Due to the highly oxidised nature of PCE and TCE, neither are considered primary substrates for aerobic microbial degradation. However, as the number of chlorine substituents in a chlorinated ethene decreases, the tendency for it to undergo oxidation increases. Hence, the aerobic degradation of DCE and VC has been demonstrated (Mattes *et al*., 2010).

The metabolites formed during the degradation of chlorinated solvents can be used as an indicator that NA is occurring. However, some daughter products are more toxic than the parent (e.g. VC produced as an intermediate of TCE reductive dechlorination to ethene). Metabolites are often susceptible to degradation but may persist if conditions are unfavourable. Isotopic (e.g. compound specific isotope analysis [CSIA, Appendix 8]) and/or biological analyses (e.g. molecular biological tools [MBTs, Appendix 9]) can provide supporting evidence to demonstrate when metabolite degradation to benign end products is occurring.

Cometabolism. Process in which a compound is fortuitously degraded by an enzyme or cofactor produced during microbial degradation of another compound. Chlorinated solvents, PAHs and some pesticides can be degraded by cometabolism (Thornton *et al.*, 2016).

Under oxic conditions, a number of organisms have been shown to be capable of the cometabolism of chlorinated ethenes to CO_2 via a non-specific oxygenase, which oxidises chlorinated ethenes to CO_2 fortuitously (Bradley and Chapelle, 2010). This process requires the presence of oxygen as well as a primary carbon substrate to maintain the production of the oxygenase. Plumes containing both chlorinated ethenes and aromatic compounds are fairly common, and under oxic conditions, the microorganisms responsible for oxidising aromatic compounds, can co-metabolise chlorinated ethenes. However, in many field settings, contaminant plumes that contain high enough concentrations of aromatic compounds for cometabolism to occur, tend to be anoxic, as oxygen has typically been preferentially consumed during microbial respiration (Bradley and Chapelle, 2010).

Under anoxic conditions, *in situ* biotransformation of chlorinated ethene parent compounds appears to occur primarily by reductive dechlorination (Thornton *et al.*, 2016).

Fermentation. Microbial metabolism in which a particular compound is used both as an electron donor and an electron acceptor resulting in the production of oxidised and reduced daughter products. Fermentation is typically the first step in the breakdown of complex organic contaminants to simpler organic metabolites which are then used in respiration reactions (e.g. SO_4-reduction, Mn/Fe-reduction, denitrification). The presence of methane is evidence of fermentation reactions in groundwater.

A2.5.2 Estimates of Contaminant Decay Rates

The rate at which many contaminants (such as hydrocarbons and chlorinated hydrocarbons) transform within the environment is commonly described using first-order kinetics, often referred to as Single First Order (SFO) (NAFTA, 2015). SFO kinetics describes reactions in which the concentration of one component is the rate-limiting step to degradation. If the concentrations of other components are involved in the rate-limiting step, the order can change to a higher order (e.g. second order), however, this section will primarily focus on first-order kinetics (Boesten *et al*., 2006).

Use of SFO kinetics can be useful in the evaluation of attenuation processes (see also Appendix 5) occurring within groundwater on contaminated sites, such as characterising trends within contaminant plumes, and providing an estimation of the time required to reach remediation goals (Newell *et al*., 2002). They are typically used to estimate bulk contaminant attenuation rates (sum of all NA processes causing a decrease in concentration in groundwater) and contaminant biodegradation rates, according to the focus of the assessment (e.g. single versus multiple well-distance analysis) and specific calculation method used. Their use can be considered as a primary line of evidence of the occurrence and rate of NA (Newell *et al*., 2002). A number of types of rate constants are available to represent different attenuation processes (Newell *et al*., 2002):

1) **Concentration versus time** – used to estimate how quickly remediation goals will be met;
2) **Concentration versus distance** – used to estimate plume behaviour through a combination of attenuation processes through bulk attenuation rate constants; and
3) **Biodegradation rate constants** – used to characterise the effects of biodegradation on contaminant migration within models.

Concentration versus Time Attenuation Rate Constants

Concentration versus time attenuation rate constants, or point decay rate constants (K_{point}), describe the behaviour of the plume at a single point, but cannot be used to provide an indication of the distribution of contaminant mass within the groundwater system. Data acquired from a single monitoring location are plotted as the natural log versus time based on several sampling events. A rate constant is derived from the slope of the line of best fit. This calculation can be used to estimate the time required (t) to reach an end goal (C_{goal}) at that specific location within the plume using the following equation (Newell *et al*., 2002):

$$t = \frac{-ln\left[\frac{C_{goal}}{C_{start}}\right]}{k_{point}}$$ Equation A2.12

Concentration versus Distance Rate Constants

Concentration versus distance rate constants, or bulk attenuation rate constants (k), are derived by plotting the natural log of the concentration from several wells downgradient of a source zone versus the distance, and calculating the rate as a product of the slope and groundwater seepage velocity (Slope m = k/vel or k = slope m.vel). The resulting rate is characterised by the distribution of the contaminant in space at that particular time

point. However, a single plot cannot provide information on the time required to reach a remediation end goal. The rate constant derived using this method incorporates all mechanisms of attenuation for contaminants within groundwater and indicates how quickly they are attenuating outside of the source (Newell *et al*., 2002).

The following formula is used to provide an estimate of the amount of time needed for the contaminants (*t*) to meet a remediation end goal (C_{goal}) as the contaminants move downgradient (Newell *et al*., 2002):

$$t = \frac{ln\left[\frac{C_{goal}}{C_{start}}\right]}{k}$$ Equation A2.13

To calculate the distance (L) that dissolved contamination will travel over a particular amount of time (t) as they are decaying, the seepage velocity (V_s) and the retardation factor (R) can be incorporated in the following equation (Newell *et al*., 2002):

$$L = \frac{V_s}{R}.t$$ Equation A2.14

Such rate constants do not represent the contaminant biodegradation rate, and should not be used within solute transport models, as attenuation processes have already been taken into consideration.

Biodegradation Rate Constant

The biodegradation rate constant can be derived by a number of methods including calibration of a solute transport model to field data, or comparison of the transport of a tracer within groundwater to contamination.

A2.5.3 Biodegradation Research on Selected CoPC

The weight of evidence available from the published literature varies, and is likely to influence the amount of site-specific data required in the early stages of an MNA lines of evidence assessment. Figure A2.5 presents an assessment of the biodegradation rates of selected CoPC, and the size of the published, peer reviewed research literature.

A search of the published literature was undertaken using Scopus in May 2020. Search terms used were the substance name OR common acronyms, AND ‘biodegradation’ (e.g. (mecoprop OR MCPA) AND biodegradation). The number of articles identified by Scopus is recorded as the number of research publications.

The biodegradation rate is the typical reported biodegradation rate (day^{-1}) in soil or groundwater, under preferable conditions for the substance in question (e.g. under aerobic conditions for BTEX compounds, but under anaerobic conditions for chlorinated ethenes). Biodegradation rate data were collated from Environment Agency (2000), Aronson and Howard (1997) and Aronson *et al.* (1999), and should be regarded as indicative median estimates of biodegradation rate for initial assessment. Site-specific

data (i.e. the primary line of evidence) are required to demonstrate MNA at each project site.

The biodegradation rates are indicative only. CoPC will biodegrade at different rates depending on site-specific conditions (e.g. electron acceptor/donor supply; microbial preference to biodegrade more labile CoPC first; toxic effects of high contaminant/salinity concentrations etc.)

Gridlines are added to separate:

1. CoPC with a small research literature (<50 articles), Moderate (50 – 500) and large (>500 articles); and
2. CoPC that are rapidly biodegradable in the subsurface environment (equivalent first-order half-life <100 days), moderately biodegradable (100 – 365 days half-life), and slowly (or not) biodegradable (>365 days).

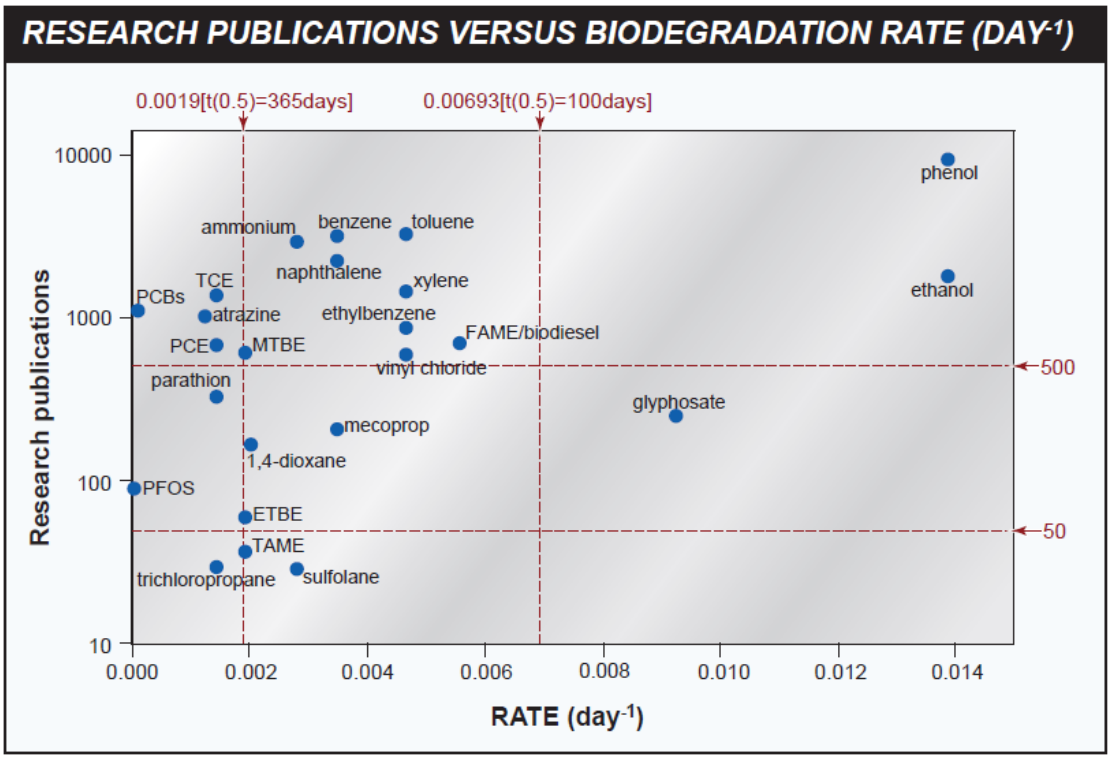


Figure A2.5: The size of the published literature on the biodegradation of selected CoPC, and their illustrative biodegradation rate in the subsurface under conducive environmental conditions.

Additional Reading

A considerable volume of published material on biodegradation and its role in MNA is available. Some selected references worthy of further reading include Thornton (2019); Rivett and Thornton (2008); Thornton *et al.* (2016); Wilson *et al.* (2004) and Ottosen *et al.* (2019). Recent research by Newell *et al.* (2021) and Ramos García *et al.* (2022) describes the science of MNA to the emerging contaminants perfluoroalkyl and polyfluoroalkyl substances (PFAS) and 1,4-dioxane respectively.

Appendix 3: Data Requirements for Lines of Evidence

Table A3.1: Parameters for MNA site characterisation and conceptual site model development.

Key lines for assessment	Applicability			Use	Potential application to NA evaluation (screening / demonstration / assessment)
	General	Organic	Inorganic	F&T = fate & transport modelling I; II; III = lines of evidence	
A. Geological and hydrogeological					
Lithology and structure	✓			F&T	Physical and geochemical properties of water-bearing units (aquifers and aquitards). Supports assessment of groundwater flow and plume migration, including preferential pathways.
Porosity	✓			F&T	Key property (including effective porosity) in assessing groundwater flow and contaminant transport.
Aquifer hydraulic conductivity, gradient & groundwater flow direction	✓			F&T	Essential for groundwater contaminant plume studies, including estimates of bulk attenuation rate, degradation rate and mass discharge.
Seasonal water level fluctuations	✓			F&T	Determines extent of smear zone and whether groundwater velocity and direction vary according to seasons. Note tidal influences and surrounding abstraction points can have impacts on temporal fluctuations.
Rates of recharge	✓			F&T	Factor in groundwater transport and input to numerical models.
B. Chemical					
Parent and daughter contaminant concentrations		✓		I	Provides a measure of the type and quantity of parent and biogenic daughter products. Used to estimate biodegradation kinetics such as half-life or degradation rate constants.
Co-contaminant concentrations		✓	✓	I	May indicate that more thermodynamically favourable degradation processes/pathways may occur, either by acting as a co-metabolite or as a catalyst.

Key lines for assessment	Applicability			Use	Potential application to NA evaluation (screening / demonstration / assessment)
	General	Organic	Inorganic	F&T = fate & transport modelling I; II; III = lines of evidence	
C. Geochemical					
Dissolved oxygen		✓	✓	II	Highest energy-yielding electron acceptor for biodegradation of organic constituents. Concentrations typically below ~0.5 mg/l generally indicate an anaerobic pathway.
Nitrate		✓		II	Thermodynamically next favourable electron acceptor after oxygen for microbial degradation of organics. Depletion may indicate (denitrification) anaerobic degradation of organics.
Nitrite		✓		II	Product of nitrate reduction, produced only under anaerobic conditions. Generally, a transient reaction by-product that is rarely detected.
Iron (III)		✓		II	Biologically available iron (III) can act as an electron acceptor during anaerobic degradation of organics.
Iron (II)		✓		II	Indication of iron (III) reduction during microbial degradation of organic compounds in the absence of oxygen, nitrate and manganese (IV) and potential for precipitation of reactive iron minerals (e.g. FeS).
Manganese (IV)		✓		II	May act as an electron acceptor during anaerobic degradation of contaminants where more thermodynamically favourable electron acceptors (e.g. oxygen and nitrate) are absent.
Manganese (II)		✓		II	Indicator of anaerobic degradation of organics, where manganese (IV) acts as an electron acceptor.
Sulfate		✓	✓	II	Used as an electron acceptor in biodegradation of organic constituents. Reduced to form sulfide.
Sulfide				II	Reduced form of sulfate indicates reduced conditions and potential for precipitation of reactive iron minerals (e.g. FeS).
Methane		✓		II	Indicator of anaerobic conditions and of degradation of organics by methanogenic bacteria and/or from biodegradation of acetate. Produced by the microbial reduction of carbon dioxide.
Ethane and ethene		✓			Metabolic end product of reductive dehalogenation of halogenated ethenes and ethane. Provides evidence of complete dechlorination of these compounds. Indicates activity of methanogenic bacteria.
Dissolved hydrogen	✓			II	Provides indicator of redox conditions, since concentrations can be correlated with types of anaerobic activities (methanogenesis, sulfate reduction) in anaerobic environments.

Key lines for assessment	Applicability			Use	Potential application to NA evaluation (screening / demonstration / assessment)
	General	Organic	Inorganic	F&T = fate & transport modelling I; II; III = lines of evidence	
Total organic carbon		✓		II	A measure of the total concentration of organic material (natural and anthropogenic) in water that may act as a primary substrate for biological degradation (reductive dehalogenation).
pH		✓	✓	II	Microbial activity tends to be lowered outside of a pH range of 6 to 8.5, and many anaerobic bacteria are particularly sensitive to pH extremes. Behaviour of metals influenced by pH.
Alkalinity/total inorganic carbon		✓	✓	II	Provides an indication of the buffering capacity of the water and the amount of inorganic carbon dioxide dissolved in the water. The latter increases due to biodegradation of organic compounds which often is a clear indicator of previous biodegradation of organic carbon compounds.
Eh (redox potential)		✓	✓	II	A measure of the oxidation/reduction potential of the environment. Typically ranges from +800 mV in strongly aerobic conditions to -400 mV under methanogenic conditions.
Temperature		✓	✓	II	Affects rates of microbial metabolism. Slower biodegradation occurs at lower temperatures. Also affects solubility of contaminants involved in reduction – oxidation processes.
Chloride	✓			II	Possible indicator of biological dechlorination. Used as a conservative tracer.
Electrical conductivity	✓			II	General water quality parameter, that can also be used with other water quality data to assess groundwater ionic strength, total dissolved solids and salinity.
Phosphorus		✓		II	Essential nutrient for microbial growth and biodegradation.
Volatile fatty acids		✓		II	Metabolic by-products of the aerobic degradation of BTEX and complex organic matter (e.g. landfill leachate plumes). Need to be compared to background values.
Abiotic degradation		✓	✓	III	Understand abundance and role of mineral phases in NA of metals, radionuclides, anions and specific petroleum hydrocarbons and chlorinated solvents. Mineral formation via environmental processes such as evaporation or degassing or through the presence of reactive minerals.
Carbon dioxide		✓		II	Used as an electron acceptor in methanogenic (anaerobic) degradation of organics. Also a product of the biodegradation of many organics.

Key lines for assessment	Applicability			Use	Potential application to NA evaluation (screening / demonstration / assessment)
	General	Organic	Inorganic	F&T = fate & transport modelling I; II; III = lines of evidence	
D. Biological					
Microbial counts/ biomass		✓		III	Demonstrate the indigenous microorganisms are capable of degrading contaminants, and to provide an indication of degradation potential. Also used to establish nutrient requirements and limitations.
Ribonucleic acid (RNA) probes		✓		III	Used to detect specific bacteria that degrade contaminants.
Compound Specific Isotope Analysis		✓		III	Analyses the relative abundance of various stable isotopes of the component elements of contamination to determine whether contaminant degradation is occurring, investigate the degradation mechanism and assist in identifying the contaminant source (Appendix 8).
Polymerase Chain Reaction		✓		III	Amplifies the genetic material of microorganisms to levels that can be further analysed using other techniques to detect microorganisms or target genes for contaminant biodegradation and process genetic material for use in other diagnostic tools.
Quantitative Polymerase Chain Reaction		✓		III	Quantifies a target gene based on deoxyribonucleic acid (DNA) or RNA to assess the abundance and the expression of specific functional genes, microorganisms, or groups of microorganisms responsible for contaminant biodegradation.
Microbial Fingerprinting Methods		✓		III	Differentiates, and in some cases identifies, microorganisms by unique characteristics of universal biomolecules to provide a profile of a microbial community, identify a subset of the microorganisms present and quantify living biomass.
Microarrays		✓		III	Detects and estimates the relative abundances of numerous genes simultaneously to provide a comprehensive evaluation of the microbial diversity and community composition.
Stable Isotope Probing		✓		III	Detects the presence of an added synthesised form of the contaminant containing a stable isotope (e.g. ^{13}C) to determine whether biodegradation of a specific contaminant is occurring and identify microorganisms responsible for this activity.
Enzyme Activity Probes		✓		III	Detects the transformation of surrogate compounds that resemble specific contaminants to quantify the activity of microorganisms with specific biodegradation capabilities.

Key lines for assessment	Applicability			Use	Potential application to NA evaluation (screening / demonstration / assessment)
	General	Organic	Inorganic	F&T = fate & transport modelling I; II; III = lines of evidence	
Fluorescence *in situ* Hybridisation		✓		III	Detects the presence of targeted genetic material in an environmental sample to estimate the number and/or relative activity of specific microorganisms or groups of microorganisms.
Environmental Molecular Diagnostics Sampling Methods		✓		III	Active sampling methods and passive microbial sampling devices in which subsurface microorganisms colonise a solid matrix to collect biomass from environmental media to be used in conjunction with specialised diagnostic methods.
Microcosm experiments		✓		III	Can be *in situ* or *ex situ* tests that allow a variety of amendments to be tested to stimulate bacterial degradation of contaminants of concern. Also, to examine any potential limitations on biodegradation activity related to the contaminant mixture (e.g. toxicity effects), environmental conditions in the aquifer (e.g. nutrient limitations) or obtain data on biodegradation rates (e.g. reaction kinetics).

Appendix 4: Data Acquisition

Table A4.1: Data acquisition.

Parameter	Main data sources	Comments
CoPC	• Desk study (source audit) • Site data (sample concentrations, flux meter technology, membrane interface probe [MIP] etc.)	Extent and mass discharge of CoPC and degradation products plumes as required by lines of evidence assessment
Porosity	• Laboratory measurement • Grain size • Hydraulic tests • Tracer test • Rock thin sections • Literature	Important to differentiate between total and effective porosity and saturated versus partially saturated. Multiple methods may be required for consolidated versus unconsolidated materials.
Henry's Law Constant	• Literature	
Bulk Density	• Laboratory measurement • Literature	Used in estimating Kd and other transport / attenuation factors
Clay content	• Laboratory measurement • Literature	Clay size and clay minerals
Fraction of organic carbon	• Laboratory measurement • Literature	Soil, aquifer sediment (if unconsolidated), rock core sample Used in estimating Kd and other transport / attenuation factors

Parameter	Main data sources	Comments
Sorption/partition coefficient	• Literature • Laboratory experiments • Tracers	Lithology, bulk density, pH dependent. Note competition between different species, chemical reactions, solubility, polarity, changes in media properties.
Hydraulic conductivity	• Rising/falling head tests • Packer tests • Pumping tests • Laboratory tests • Grain size-based estimates • Hydraulic Profiling Tool (HPT) • Cone Penetrometer Tool (CPT) • Literature	Hydraulic conductivity may vary laterally and vertically (heterogeneity, anisotropy). Unsaturated zone hydraulic conductivity dependent on water saturation. Preferable to obtain bulk or horizontal hydraulic conductivity from field tests. Laboratory tests are more appropriate for vertical hydraulic conductivity, lower hydraulic conductivity materials, aquitards. British Geological Survey Aquifer Properties Manuals.
Groundwater levels Hydraulic gradient	• Observation boreholes and/or monitoring wells	Locations should aim to develop CSM, with representative response zones to check preferential flow paths. Possibility of more than one hydrogeological regime (i.e. components of downward, or upward flow, aquitards, semi-confined, perched water).
Aquifer thickness Mixing depth	• Boreholes • Geophysical logging • Packer testing • CoPC distribution • Groundwater level variation	Flow may be in discrete zones such that aquifer thickness may differ from the total depth of the formation. Mixing depth can be estimated using empirical equations.
Aquifer geometry	• Geological maps • Boreholes and monitoring wells • Geophysical survey • Core retrieval • Fracture conditions (aperture size, infill orientation etc.)	The hydrogeological framework model is fundamental to developing a defensible CSM for NA.

Parameter	Main data sources	Comments
Aquifer geometry (cont.)	• Interbedded and stratified depositional understanding to inform groundwater regimes • Sequence stratigraphy	
Direct recharge	• Climatological data (rainfall, evaporation) • Land-use, land surface • Soil type	Variable recharge through complex depositional environments (including low permeability drift) and deposits of anthropogenic origin.
Indirect recharge (leakage or discharge to sewers, drains, water mains)	• Flow gauging • Desk study / utilities mapping	Include these locations/features in plots of field data to contour hydraulic gradients. Unusual variation in local gradient may be indicative.
Receptors	• Environment Agency and Natural England, Scottish Environment Protection Agency and NatureScot, Natural Resources Wales, Northern Ireland Environment Agency, Environmental Health Departments	Should include site inspections and walkovers
Abstraction rates	• Environment Agency, Scottish Environment Protection Agency, Natural Resources Wales, Northern Ireland Environment Agency, local authorities	Actual abstraction may not equal the licensed abstraction rate.
Dispersion coefficient	• Empirical values (one tenth of distance plume has migrated) • Model calibration • Tracer studies • Literature	The value of the dispersion coefficient is scale-dependent. Values reported in field experiments are often several orders of magnitude greater than from laboratory experiments.

Parameter	Main data sources	Comments
Aquifer mineralogy	• Mineralogical analysis (x-ray diffraction, sequential extractions etc.) • Literature	Distribution and abundance of mineral phases important for understanding metal and anion transport, plus reactive minerals involved in biodegradation or abiotic degradation (e.g. solid phase electron acceptors [Fe, Mn oxides, carbonates] and reactive FeS minerals).
Natural Source Zone Depletion (NSZD)	• Soil gas, vapour and temperature measurement, CoPC source mass discharge in groundwater, LNAPL compositional change	For petroleum hydrocarbon LNAPL, see also Technical Bulletin 20 (CL:AIRE, 2019a) and emergent NSZD good-practice guidance from CL:AIRE (2024).
NAPL properties & distribution	• Density, viscosity, interfacial tension, boiling point, composition analysis (e.g. fingerprinting, individual components), age determination, LNAPL mobility assessments. NAPL dye testing, tracer tests, bail down tests, Flexible Liner Underground Technologies (FLUTe) liners, ultra violet optical screening tool (UVOST), tar-specific green optical screening tool (TarGOST), dye-laser induced fluorescence (Dye-LIF)	See also the Illustrated Handbook of DNAPL Transport and Fate in the Subsurface (Kueper *et al.*, 2003), the Illustrated Handbook of LNAPL Transport and Fate in the Subsurface (CL:AIRE, 2014) and emergent NSZD good-practice guidance from CL:AIRE (2024).
Biodegradation	• Analysis of observed changes in contaminant concentrations • Microbiological studies • Literature • Lab & *in situ* microcosms, bio-traps, compound specific isotope analysis, microbial cell presence, abundance and functional gene analysis, polymerase chain reaction (PCR), quantitative polymerase chain reaction (qPCR)	Breakdown products with different properties. Consideration of the biogeochemical environment. Typically represented as first or second-order decay kinetic reaction. Alternatively, may be linked to available terminal electron acceptor process indicator parameters (oxygen, nitrate, sulfate, ferrous iron, manganese II, methane). Geochemical, isotopic and microbiological sampling in groundwater often requires specific sample handling and preservation (see BS EN ISO 5667-3 [British Standards Institution, 2018], and laboratory/analytical method specific requirements).

Appendix 5: Methods of Assessment

A5.1 Introduction

This appendix provides supporting information on some of the methodologies, tools and visualisation techniques that are available to assist with the assessment and demonstration of NA and degradation rates.

In 2004 the British Geological Survey (Lelliott and Wealthall, 2004) undertook a review of the qualitative, quantitative and visual means of describing the evidence for NA. This was organised according to the lines of evidence approach that is central to the demonstration of NA with additional explanatory discussion and some visualisation techniques for data. The reader is referred to that report for detailed descriptions of the methodologies described here. This revised appendix also incorporates advances in data presentation as appropriate from a number of other sources. The methods presented are not exhaustive, but are believed to represent the principal methods employed.

A5.2 Primary Lines of Evidence

A5.2.1 Graphical Techniques

Evidence for natural attenuation can be obtained by comparing contaminant concentrations or ratios along the groundwater flow path where the change in solute concentration in the groundwater over time often can be described using a first-order decay rate constant (Lelliott and Wealthall, 2004; Rivett and Thornton, 2008). Examples of these type of plots include:

- monitoring well concentration plots (concentration versus time) (Figure A5.1) in which concentrations of CoPC are analysed over time in a single well to identify trends at one point in the plume. These data are not representative of the plume as a whole but can provide a useful indication of temporal behaviour in a particular location and can be combined with other locations;
- plume centreline concentration plots (concentration versus distance) in which the change in CoPC concentrations along the centreline of a plume for a given time period (i.e. specific groundwater sampling event) are plotted (Figure A5.2). Critical in this instance are a series of monitoring locations that are aligned along the centreline of a plume and a hydrogeological regime that does not have widely variable flow directions over the course of monitoring. This method is only really applicable to analysis of stable or shrinking plumes. The data can also be difficult to interpret where the geology / hydrogeology is complex and non-uniform along the centreline and the centreline itself may be difficult to establish; and
- comparison of contaminant ratios where plots can include log-normalised concentrations of contaminants with distance, and ratios of contaminant concentrations with distance. Comparison of normalised concentrations for a conservative contaminant to other contaminants can be used to identify different

rates in migration due to sorption or degradation (Figure A5.3). For these purposes conservative may be defined as a non-retarded, non-reactive contaminant that is spilled at the same time or location, or a similar contaminant within the spillage that is not readily biodegraded but has similar physical characteristics (Lelliott and Wealthall, 2004). The use of contaminant ratios can be complicated by the presence of multiple sources and background concentrations requiring subtraction.

It should be noted that concentration data can be mass or molar concentrations. Molar concentrations are particularly useful for comparing parent with degradation product (daughter) concentrations.

The above plots can be coupled with calculations of statistical parameters including the slope of the line of best fit, coefficient of variation (COV), r^2 value, and confidence levels. Such functionality is available in a number of publicly available domain software packages (e.g. MAROS & GWSDAT).

Examples of each of the three techniques are presented in Figures A5.1 to A5.3 (Lelliott and Wealthall, 2004).

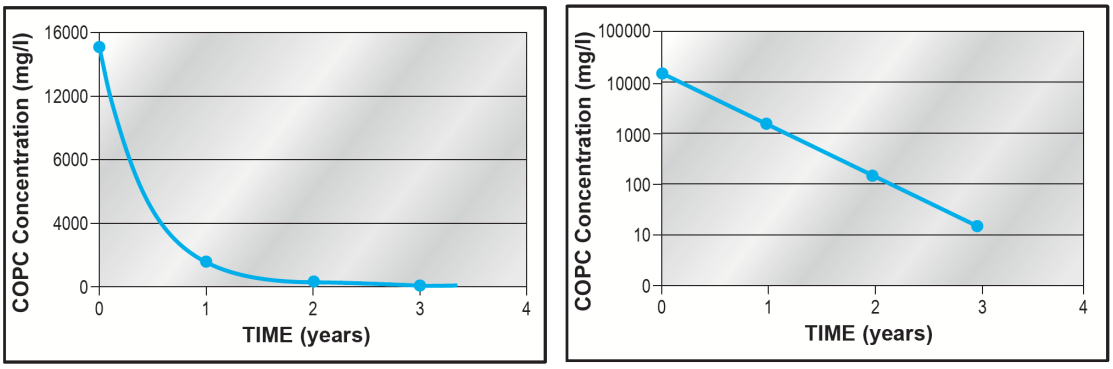


Figure A5.1: First-order decay for contaminants of concern for a single monitoring well/location. Reproduced from Lelliott and Wealthall (2004) with permission from the British Geological Survey © UKRI 2004 (BGS permit no. CP23/057).

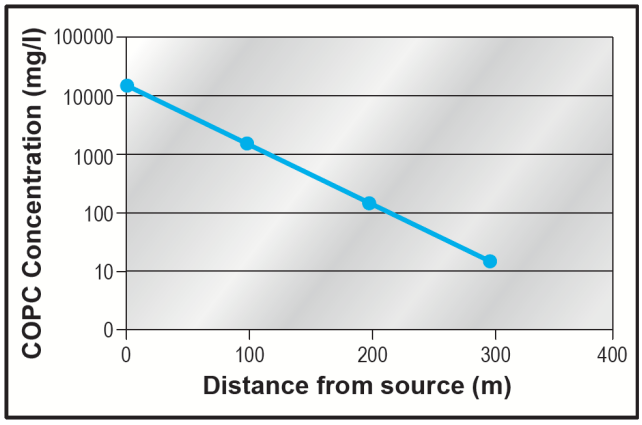
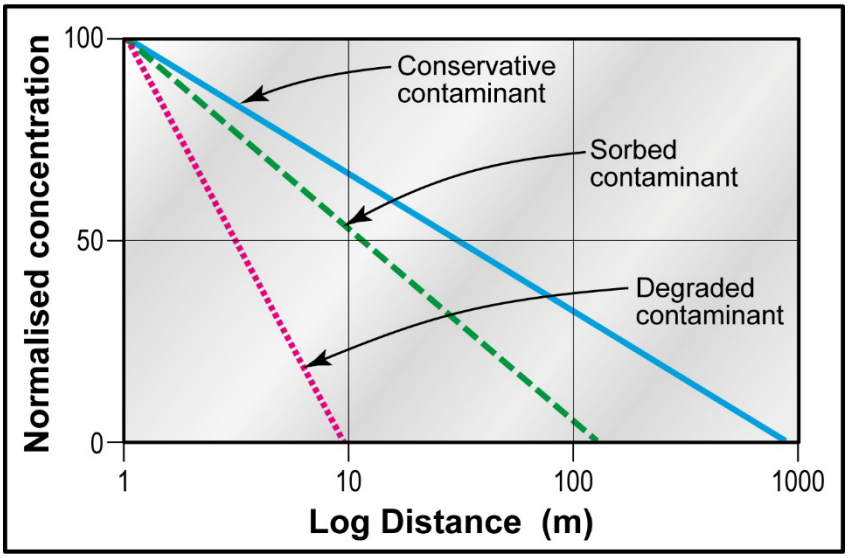


Figure A5.2: Centreline concentration plot for average contaminant of concern concentrations for a stable plume, or individual monitoring event for a shrinking plume. Reproduced from Lelliott and Wealthall (2004) with permission from the British Geological Survey © UKRI 2004 (BGS permit no. CP23/057).



Notes

- This plot is created by normalising the concentration of a solute at successive distances along the plume flow path to the source concentration of that solute.
- This plot compares the attenuation of reactive solutes (e.g. by sorption or degradation) relative to that of a conservative species, which is assumed to decrease in concentration along the flow path by dilution due to dispersion.
- The form of this plot allows (i) different rates of contaminant migration due to sorption to be deduced for contaminants which are known or assumed to be recalcitrant under the given conditions, (ii) different rates of degradation to be deduced for contaminants with similar sorption characteristics.

Figure A5.3: Comparison of contaminant concentrations normalised to source term concentration estimate (i.e. C/C_o) (after Environment Agency, 2000).

A5.2.2 Visual Techniques

Plume contour plots

Plume contour plots in either plan view or as a cross-section through the centreline of the plume are the most common visual method to identify whether a plume is stable, shrinking, or expanding over time (Lelliott and Wealthall, 2004; Rivett and Thornton, 2008). Contour plots can (and should) also be created to show the spatial-temporal distribution of biogeochemical indicator species and compared with the contaminant contour plots for proper interpretation of the plume dynamics and associated degradation processes. Cross-section contour plots are typically orientated along the centreline of the plume and are used to give an indication of the vertical variation in contaminant concentration. The latter is only applicable where suitable multi-level monitoring wells, or nested groups of monitoring wells with differing depths are positioned in the plume.

In each of the above instances, visual examination over different time intervals can give an immediate impression of the plume status and can also be used to compare the plume shape and orientation to the groundwater flow direction and/or modelled predictions, as shown in Figure A5.4

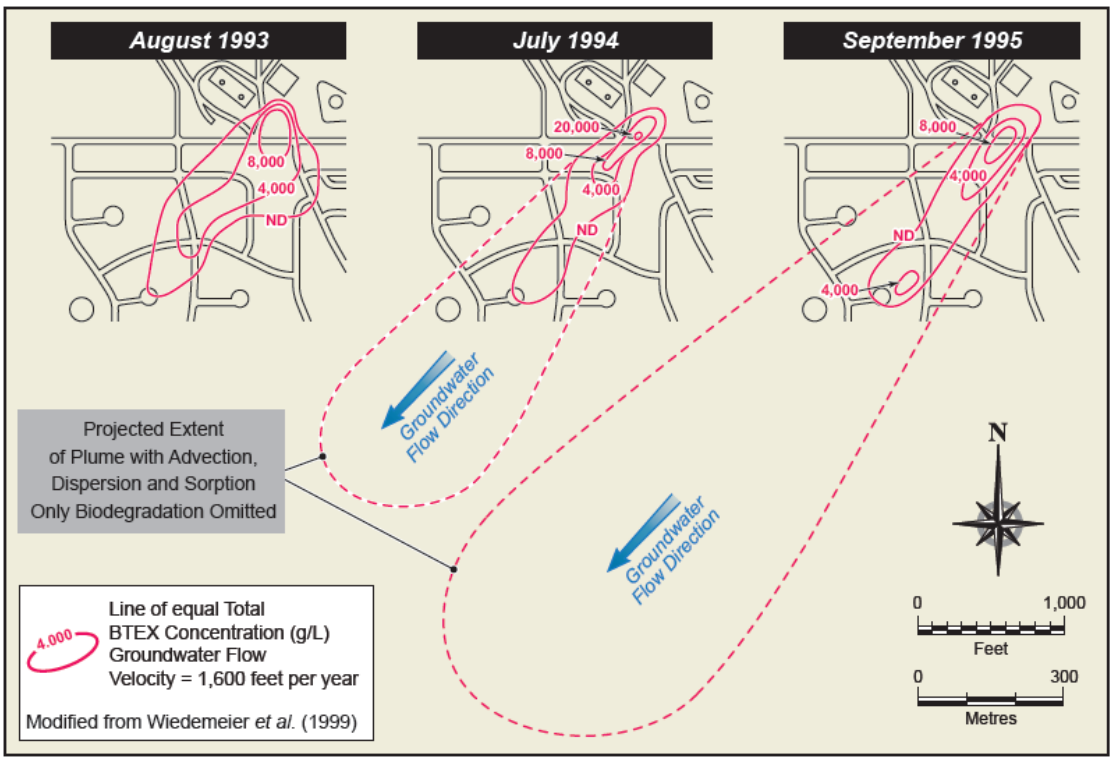


Figure A5.4: Comparison of projected vs actual plume migration (Wiedemeier *et al.*, 1999). © 1999 John Wiley & Sons, Inc.

2D visualisation or 3D interpolation software is widely available (e.g. Surfer, Earth Volumetric Studio [EVS], Leapfrog, Spatial Analysis and Decision Assistance [SADA], GWSDAT and RockWorks) and 2D and 3D contouring and gridding may be used to construct contour maps and with 3D packages cross-sections along one or numerous planes to visualise the plume. Visual analysis of plume orientation can also be coupled with quantitative measures of a number of plume characteristics (Figure A5.5) including plume area, average concentration, plume mass and centre of mass (Ricker, 2008) and this functionality is included in GWSDAT (Figure A5.6) and MAROS (Aziz *et al.*, 2000).

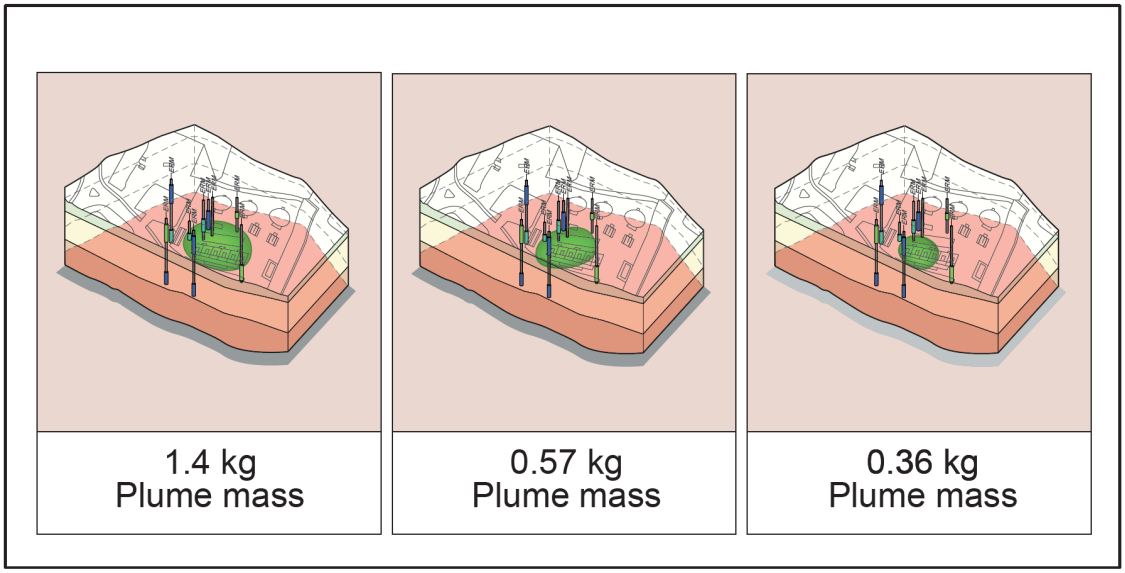


Figure A5.5: Decrease in dissolved plume mass over time for a chlorinated solvent plume undergoing NA (Source: ERM).

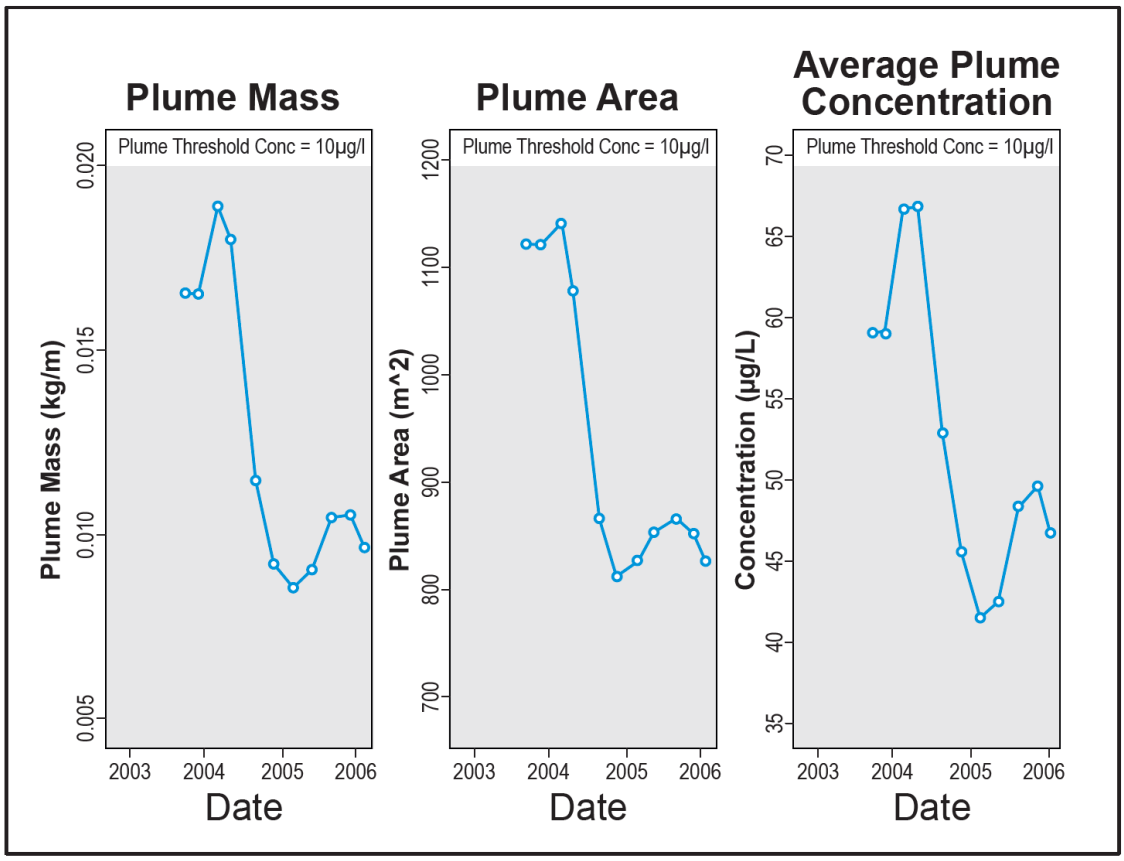


Figure A5.6: Example of the summary output of plume metrics from GWSDAT: plume mass (left), plume area (mid) and average concentration (right) (CL:AIRE, 2019b).

The creation of a contour plot or 3D visualisation requires interpolation (e.g. kriging) of the chemical distribution between monitoring wells (Lelliott and Wealthall, 2004) and care should be undertaken in interpretation. Contour plots and 3D models should be used with caution where there is not a high-density monitoring network or where the geology / hydrogeology is spatially variable (Wilson *et al.*, 2004), care should be taken on inappropriate integration of the vertical, with well screens say at different depths / in different units shown on the same spatial plot that may not always be appropriate and misleading. In addition, during the course of an MNA study whatever the extent of data available it is equally or more important the monitoring points and data analysis are kept consistent (assuming the CSM does not change – e.g. if flow direction changes in response to abstraction) to ensure that any changes observed are all being measured to the same baseline and context. Any unavoidable changes should be phased in gradually wherever possible. For example, if monitoring boreholes require relocation if neighbouring land ownership changes then new boreholes should be installed ahead of decommissioning old ones so there is overlap in dataset to allow comparison.

A5.2.3 Statistical Techniques

In addition to or as a supplement to the above, statistical procedures and models can provide a formal, quantitative method for assessing plume stability (NJDEP, 2012).

The need for application of statistical tests and the nature of the tests will vary as a function of site-specific conditions and data analysis requirements (UK TAG, 2012). The methods must be appropriate for undertaking the trend assessment and be applicable

to the available data. Groundwater quality data possess unique characteristics that require specialist approaches to statistical testing. Groundwater data often have asymmetric or non-normal distributions. These 'skewed' datasets may therefore require use of alternative non-parametric statistical methods where no assumptions are required about the underlying data distribution (UK TAG, 2012). Alvarez and Illman (2005) indicate that the Mann-Kendall Test (including the Seasonal Kendall) and the Mann-Whitney U Test are widely applied.

Mann-Kendall Test

The Mann-Kendall analysis is a non-parametric statistical procedure that is used to statistically assess if there is a monotonic upward or downward trend of the variable of interest over time. A monotonic upward (downward) trend means that the variable consistently increases (decreases) through time, but the trend may or may not be linear.

The Mann-Kendall test neither requires a specific statistical distribution of the data, nor is the test sensitive to the sampling interval over which the monitoring data are collected. The outcome of the procedure depends on the ranking of individual data points and not the overall magnitude of the data points. Therefore, the Mann-Kendall procedure can be used for datasets that include irregular sampling intervals, data below the detection limit, and trace or missing data. The approach is particularly advantageous in cases where outliers in the data could produce biased estimates using parametric trend analysis (GSI, 2012).

The Mann-Kendall test for trend analysis is available within a number of public domain tools both as a component of broader packages (MAROS, GWSDAT) but also as a standalone tool (e.g. GSI Mann-Kendall Toolkit [GSI, 2012]). The latter includes three statistical metrics (GSI, 2012) as follows:

- The 'S' Statistic: Indicates whether concentration trend versus time is generally decreasing (negative S value) or increasing (positive S value).
- The Confidence Factor (CF): The CF value modifies the S Statistic calculation to indicate the degree of confidence in the trend result, as in "Decreasing" versus "Probably Decreasing" or "Increasing" versus "Probably Increasing." Additionally, if the CF is quite low, due either to considerable variability in concentrations versus time or little change in concentrations versus time, the CF is used to apply a preliminary "No Trend" classification, pending consideration of the COV.
- The Coefficient of Variation (COV): The COV is used to distinguish between a "No Trend" result (significant scatter in concentration trend versus time) and a "Stable" result (limited variability in concentration versus time) for datasets with no significant increasing or decreasing trend (e.g. low CF).

The rules applied by the GSI Mann-Kendall Toolkit to classify plume concentration trends were developed based upon empirical analysis of hundreds of groundwater plumes (GSI, 2012).

An example of the calculation of the S Statistic is provided below in Table A5.1 and Table A5.2 provides a summary of the statistical approach.

Table A5.1: Example of S Statistic calculation (after GSI, 2012).

Sample Event Number	1	2	3	4	5	
Benzene concentration (mg/l)	13.95	42.08	33.9	33.67	18.05	Total Points
Comparison to event 1		+1	+1	+1	+1	+4
Comparison to event 2			-1	-1	-1	-3
Comparison to event 3				-1	-1	-2
Comparison to event 4					-1	-1
		Apparent Decreasing Trend			S=	-2

Table A5.2: Example statistical metrics used in GSI Mann-Kendall Toolkit (Aziz *et al.*, 2003). © 2003 John Wiley & Sons, Inc.

S Statistic	Confidence in Trend	Trend
S > 0	CF > 95%	Increasing
S > 0	95% ≥ CF ≥ 90%	Probably Increasing
S > 0	CF < 90%	No Trend
S ≤ 0	CF < 90% and COV ≥ 1	No Trend
S < 0	CF < 90% and COV < 1	Stable
S < 0	95% ≥ CF ≥ 90%	Probably Decreasing
S < 0	CF > 95%	Decreasing

Note: CF=Confidence Factor; COV=Coefficient of Variation. The user can identify two other categories of Data: ND=Dataset where all values are non-detect, and N/A=locations with <4 sample results.

Mann-Whitney U test

The Mann-Whitney U test is another statistical test that may be useful at a site. The outcome of the test is not influenced by the overall magnitude of the data, but rather is based on the ranking of individual data points.

The test is conducted by vertically ranking the eight data points from lowest to highest, with the lowest value on top and greatest value on the bottom. For each individual "A" concentration, the number of "B" concentrations that occur below the "A" concentration are counted. The four values (either zero or some positive number) are summed together

to obtain the U statistic. All non-detect values are considered zero. If two or more concentrations are identical, then two vertical columns are constructed. In the first column, the tying "B" concentration is ranked first, and in the second column the tying "A" concentration is ranked first. An interim U is calculated for each column, and the average of the interim U values is used as the final U value. If U = 3 then the null hypothesis is rejected, and it is concluded with at least 90% confidence that the concentration for the individual contaminant at that well has decreased over time. If U > 3, the null hypothesis is accepted, and it cannot be concluded with at least 90% confidence that the concentration for the individual contaminant has decreased with time at that well (Wiedemeier *et al.,* 1999).

In many groundwater systems there will be considerable seasonal variability in parameter concentrations. This variability may introduce problems in the trend analysis unless it can be corrected for. Where there are sufficient data within a given year, the best way to do this is to fit a seasonal model to the data and then use this to "de-seasonalise" the data. The Seasonal Kendall test is a modification of the Mann-Kendall test that addresses short-term seasonal variability and allows evaluation of overall trends. In a Seasonal Kendall test, the Mann-Kendall test is applied to each season (e.g. quarter) separately and then the results are combined for an overall test (NJDEP, 2012). The alternative, where there are variable or insufficient within year measurements, is to remove seasonality by calculating the annual means, and then to perform the trend analysis on the annual means. In this case Sen's Method has been recommended (NJDEP, 2012). Both the Seasonal Kendall test and Sen's Method are robust methods that allow for some missing data in the time series and are not badly affected by gross errors or outliers in the data series.

crcCARE (2010) notes that at very low concentrations, these tests may be difficult to apply, and selection of sampling data is important and to avoid biasing these statistical tests, the same number of significant figures should be consistently used for a given contaminant. This ensures that any plume trends are true data trends and not an artefact of laboratory reporting formats (crcCARE, 2010).

A5.3 Secondary Lines of Evidence

A5.3.1 Natural Attenuation Rates

The use of first-order attenuation rate constants in NA studies has been described in detail by Newell *et al.* (2002). Rate calculations based on the graphical methods outlined in Section A5.2.1 for primary lines of evidence (well concentration plots and centreline concentration plots) can be used as part of MNA studies to evaluate the contribution of attenuation processes and the anticipated time required to achieve remediation objectives. Note that these calculations are most easily applied where contaminant concentrations are quite high and may be more difficult to interpret at plume margins where lower concentrations may be at or near the limit of quantification as patterns can get lost in 'noise' in the dataset. Table A5.3 describes each of the rate constants and summarises the potential uses of each in NA studies. This is followed by a brief description of each.

Table A5.3: Summary of first-order rate constants for NA studies (Newell *et al.*, 2002).

Rate Constant	Method of Analysis	Significance	Use Rate of Constant		
			Plume Attenuation	Plume Trends?	Plume Duration?
Point Attenuation Rate (K_{point}, time per year)	C vs T Plot	Reduction in contaminant concentration over time at a single point	NO*	NO*	YES
Bulk Attenuation Rate (k, time per year)	C vs D Plot	Reduction in dissolved contaminant concentration with distance from source	YES	NO*	NO
Biodegradation Rate (λ time per year)	Model Calibration, Tracer Studies, Calculations	Biodegradation rate for dissolved contaminants after leaving source, exclusive of advection, dispersion, sorption etc	YES	NO	NO

*Note: Although assessment of an attenuation rate constant at a single location does not yield plume attenuation information, or plume trend information, an assessment of general trends of multiple wells over the entire plume is useful to assess overall plume attenuation and plume trends.

Point attenuation rate constant

The point attenuation rate constant (K_{point}) uses contaminant concentrations with time for a monitoring well located within the plume. The rate constant is calculated by plotting the natural log of a concentration against time at a particular monitoring point (Figure A5.7). This rate is the result of the combined effects of dispersion, biodegradation, and other attenuation processes (Newell *et al.,* 2002).This method is only applicable to shrinking plumes (ASTM, 1998), if the plume is stable then K_{point} will be very small.

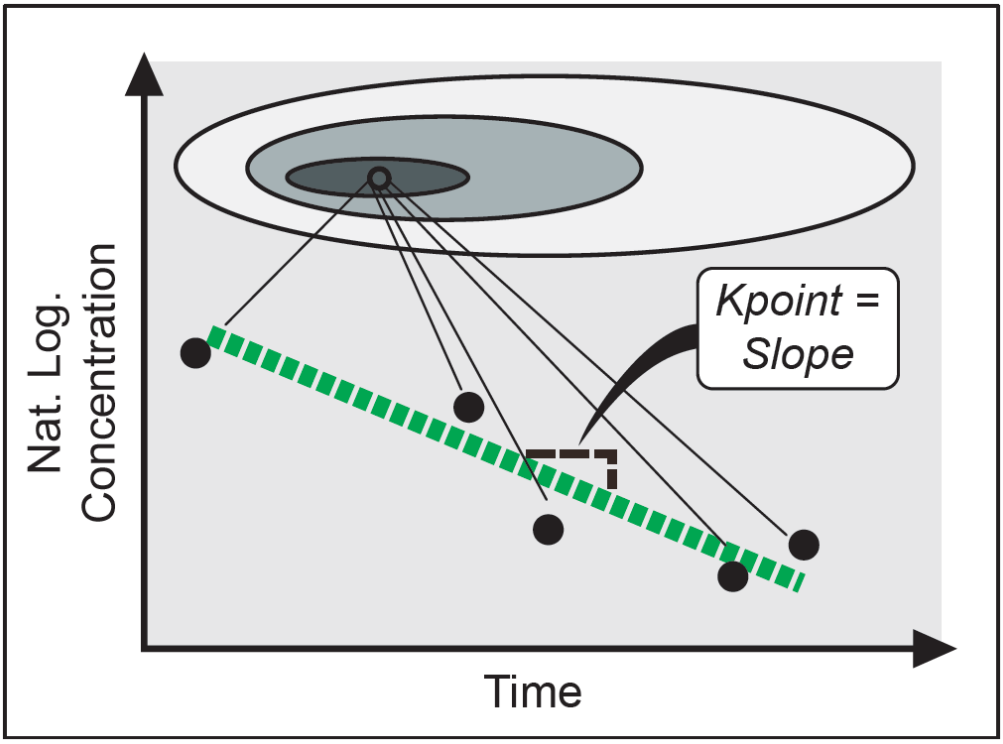


Figure A5.7: Determination of concentration versus time rate constant (K_{point}) (after Newell *et al.*, 2002).

A rate constant derived from a well concentration plot provides information regarding the potential plume lifetime, or time to reach a remedial target, at that location, but cannot be used to evaluate the distribution of the contaminant mass within the groundwater system (Newell *et al.,* 2002). The entire plume can be assessed by determining rate constants in a number of monitoring wells throughout the plume (Lelliott and Wealthall, 2004).

Bulk attenuation rate constant (k)

The bulk attenuation rate constant (k) uses contaminant concentrations with distance along the centreline of the plume for a given time period. The constant is derived by plotting the natural log of the concentration versus distance and (if determined to match a first-order pattern) calculating the rate as the product of the slope of the transformed data plot and the groundwater seepage velocity (Figure A5.8). Degradation typically occurs as a first-order rate reaction and would be expected to plot as a straight line on a log-linear plot (ASTM, 1998). The rate constant calculated using this methodology is due to the combined effects of dispersion, biodegradation, and other attenuation processes (Newell *et al.,* 2002). This technique is only applicable to stable or shrinking plumes.

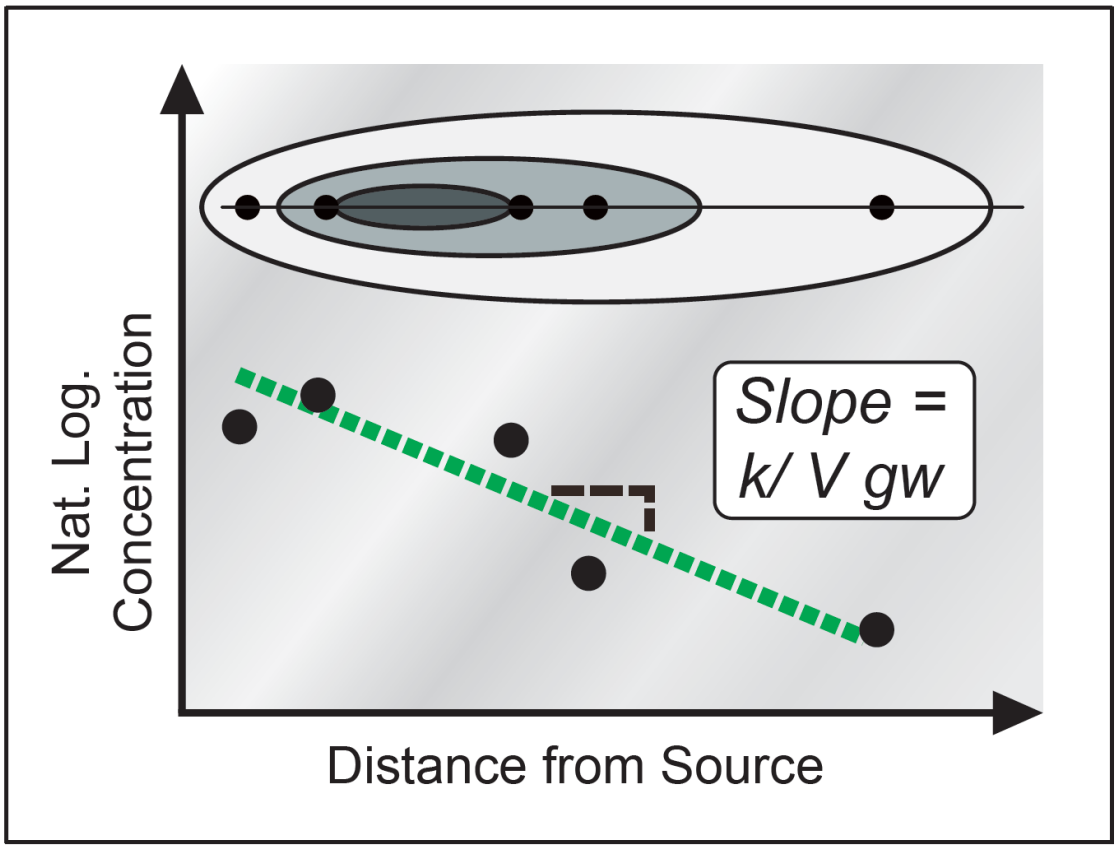


Figure A5.8: Determination of concentration versus distance rate constant (k) (after Newell *et al.,* 2002).

Biodegradation rate constant (λ)

The biodegradation rate constant (λ) is a component of the bulk attenuation constant described above and determines the portion of the overall attenuation that can be attributed to biodegradation. The biodegradation rate constant can be determined by:

- comparing the contaminant concentration along the flow path with a conservative contaminant (non-degraded), referred to as a conservative tracer;
- using the methodology derived by Buscheck and Alcantar (1995) that identifies the contribution of biodegradation for a steady-state plume by coupling the regression of contaminant concentration versus distance downgradient (centreline concentration plot) to an analytical solution for one-dimensional, steady-state, contaminant transport that includes advection, dispersion, sorption, and biodegradation (Figure A5.9); and
- be calculated by calibration of a solute transport model to field data.

The principles of each are briefly described below. Any type of rate constant calculation should be verified by observed groundwater concentrations during the performance monitoring period.

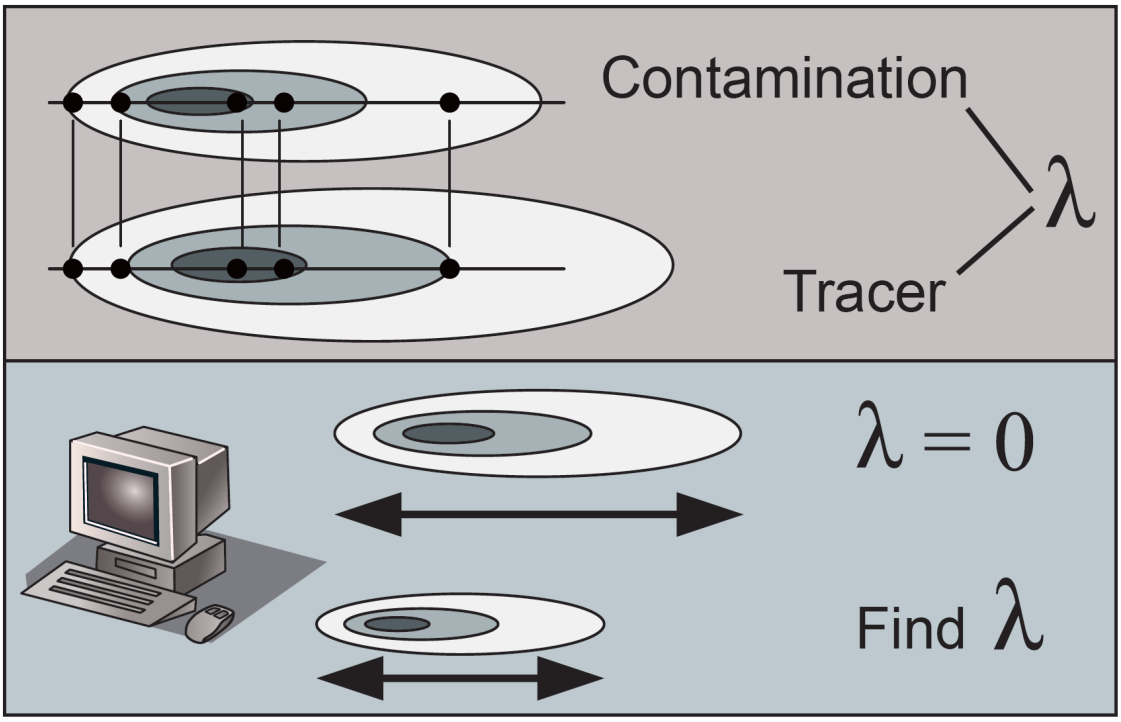


Figure A5.9: Determination of biodegradation rate constant (λ) (after Newell *et al.*, 2002).

The use of conservative tracers to calculate biodegradation rate constants

For a tracer to be useful, it will need to be biologically recalcitrant and have similar Henry's Law constant and soil sorption coefficients to the contaminant of interest, or be subject to less retardation than the contaminant(s) of concern. The tracer will also normally be associated with the original contaminant spill. Examples of a conservative tracer include:

- chloride or bromide (both examples of non-sorbing and non-degrading tracers), if released within the original spill;
- trimethylbenzene (TMB) and tetramethylbenzene, (that are sorbing but more recalcitrant) which are typically present in fuel mixtures, although under certain conditions these organics can be degraded.

The concentration of a contaminant at a point (B) downgradient of the source (A) can be corrected for the effect of dispersion, dilution and sorption using Equation A5.1:

$$C_B C_{orr} = C_B \left(\frac{T_A}{T_B}\right) \qquad \text{Equation A5.1}$$

Where:

$C_B C_{orr}$ = corrected concentration of contaminant at point B [M/L^3]

C_B = measured concentration of contaminant at point B [M/L^3]

T_A = measured concentration of tracer at point A [M/L^3]

T_B = measured concentration of tracer at point B [M/L^3]

However, for conservative tracers, the following need to be demonstrated:

- the tracer is recalcitrant; and
- the tracer behaviour is otherwise similar to the contaminant and was released at the same time and location as the contaminants of concern.

By plotting corrected contaminant distribution on a log-linear plot of corrected concentration against downgradient travel time along the flow path the degradation rate can be calculated using Equation A5.2:

$$\lambda = -\frac{1}{t} \ln \frac{C_B}{C_A}$$ Equation A5.2

Where:

λ = first-order degradation rate [t^{-1}]

C_B = tracer-corrected contaminant concentration at time *t* at downgradient point B

C_A = measured contaminant concentration at upgradient point A

t = travel time between points A and B where $t = x/u$ (*x* = distance between A and B, *u* = retarded solute velocity due to sorption)

In reality (field conditions) this requires careful planning and execution in all but the simplest groundwater systems. Calculation of biodegradation rates using the above method can be difficult in complex groundwater systems but remains a useful technique to consider.

Determination of degradation rate for a steady state plume

For a steady-state plume, the first-order decay rate is given in Equation A5.3 (Buscheck and Alcantar, 1995):

$$\lambda = \frac{V_c}{4\alpha_x}\left[[1 + 2\alpha_x]\left[\frac{k}{v_x}\right]^2 - 1\right]$$ Equation A5.3

Where:

λ = first-order biological decay rate [t^{-1}]

V_c = retarded contaminated velocity (due to sorption) in the x-direction [Lt^{-1}]

α_x = longitudinal dispersivity [L]

k/v_x = slope of line formed by making a log linear plot of contaminant concentration versus distance downgradient along flow path, and where v_x is the groundwater flow velocity

Application of analytical and numerical models

The first-order biodegradation rate can also be calculated by calibration of a solute transport model to field data (Newell *et al.,* 2002). Models that can be used include BIOSCREEN, BIOCHLOR, BIOPLUME III, and MT3D, however it is necessary to ensure that the lines of evidence are available to substantiate the derived biodegradation rate and that the biodegradation rate has not been derived purely to fit the model (other variables may be wrongly measured or estimated) (Lelliott and Wealthall, 2004). For chlorinated solvents, models with the ability to stimulate reductive dechlorination as a sequential first-order decay process should be utilised. Sequential first-order decay means that a parent compound undergoes first-order decay to produce a daughter product and that product undergoes first-order decay and so on. An illustration of the behaviour of TCE and the production of associated daughter products is presented in Figure A5.10.

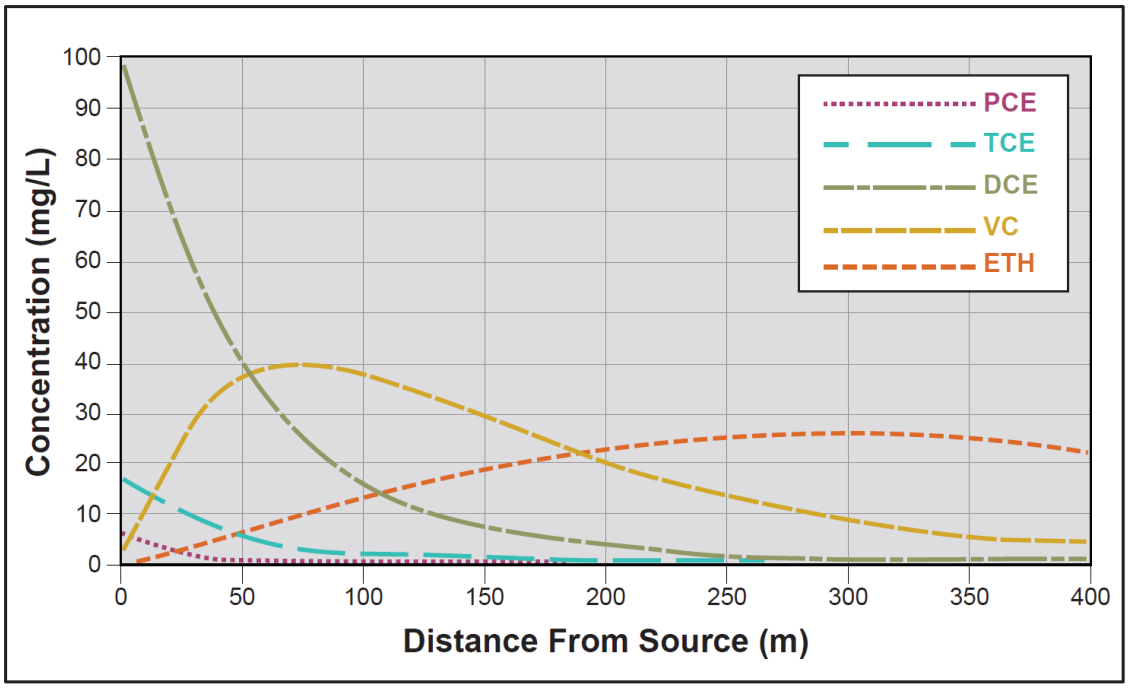


Figure A5.10: Reactive transformation of chlorinated ethenes (adapted from Aziz *et al.*, 2000).

The role of sequential degradation in the MNA of chlorinated solvents is described in detail elsewhere (Wiedemeier *et al.*, 1998) together with methodologies and approaches for incorporating the degradation rates of intermediate products (Aziz and Newell, 2002). The further application of models to demonstrate MNA is addressed in Appendix 7 – Groundwater Flow and Transport Models.

It is also recommended that:

- In this form of simulation model results should be compared with field data (Figure A5.11) and the model parameter values adjusted to obtain a model fit with the observed data.
- The final model parameter values should be assessed for reasonableness. For example, if the analysis indicates a degradation rate with a half-life of five days, whilst literature values for similar sites indicate values of 100 to 1000 days are more appropriate, then the assessment should be critically re-evaluated.

- Finally a sensitivity analysis should be undertaken to determine which parameters have the greatest influence on the model results and assess whether further data are required.

Overall the analysis should be reviewed in terms of:

- uncertainty in understanding of the system and in the conceptual model;
- uncertainty in parameter values; some may vary by more than an order of magnitude;
- applicability of the model to the site (including model assumptions);
- use and relevance of literature values to define model parameters; and
- whether there is more than one solution, that is whether different combinations of parameter values can give the same result.

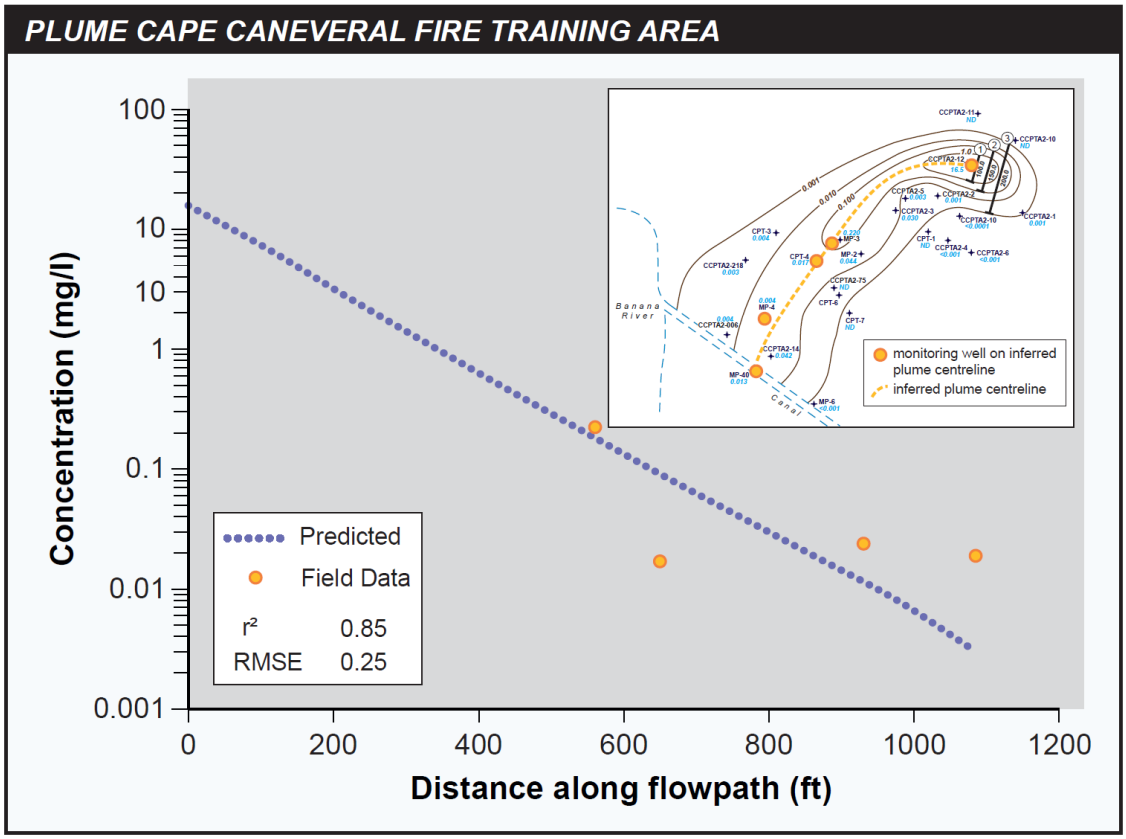


Figure A5.11: Graph adapted from the Cape Canaveral TCE plume case study in the BIOCHLOR manual (https://nepis.epa.gov/Adobe/PDF/P1000YUW.pdf). r^2 is the coefficient of determination and RMSE is the Root Mean Square Error. The inset map shows plume concentration contours with observed TCE concentrations with an overlay showing an inferred plume centreline and monitoring wells used in the 1D BIOCHLOR model. This example is effectively the same as the bulk attenuation rate (k) analysis (Figure A5.8).

Mass balance methods

The primary line of evidence in NA studies is the documented loss of contaminant mass in the field (such as historic data showing a reduction in contaminant concentrations with time). The quantification of reduction in contaminant mass within a plume or across a defined boundary over time can therefore form an important part of the overall lines of evidence for NA at a given site.

Calculation of mass in dissolved phase plume

The mass of contaminant in a dissolved phase plume can be estimated using Equation A5.4:

$$Dissolved\ mass(M) = C_{av}\ b\ n\ A \qquad \text{Equation A5.4}$$

Where:

M = dissolved mass [M]

C_{av} = average plume concentration [M/L^3]

b = aquifer or plume thickness [L]

n = porosity

A = plume area [L^2]

Though a relatively simple equation, care is required in a number of key assumptions:

- determining the area occupied by the plume – this could include the area above detection limits or the area above regulatory guidance concentrations, which may be defined as a single area or could include the preparation of contaminant concentration contours and calculating the area between each contour;
- determining the thickness of the plume, this is ideally based on and defined by, non-detection of contaminants within the monitored profile and can be established from multilevel monitoring wells or the results of a high-resolution site characterisation but can also be estimated from traditional monitoring wells or from calculation of the mixing zone (crcCARE, 2010).
- determining the average concentration in the plume – this may be calculated from (crcCARE, 2010):
    - the calculated arithmetic or geometric mean of all concentrations inside the defined overall area of the plume;
    - the calculated arithmetic or geometric mean between each contaminant concentration contour; or
    - using kriging or interpolation software.

2D and 3D contouring and gridding using commercially-available packages may be used to construct contour maps and with 3D packages a volume estimate to allow estimation of mass (see Figure A5.5 in Section A5.2.2) and are subject to the same assumptions and limitations as observed for the production of visual graphics (and may be constrained by the complexity of the hydrogeological setting).

Though simple to undertake, the calculation of contaminant mass in the dissolved phase plume can be subject to considerable uncertainty that may over or underestimate mass depending on a number of variables including:

- The lateral and vertical delineation of the plume and mixing zone (geological heterogeneity and resolution, number of wells, spatial distribution, screen length, single or multilevel monitoring well);
- The need for and consistency in appropriate groundwater sampling methods to ensure data quality;
- The consistency in monitoring over a period of time (consistency in groundwater flow direction, water table elevation, groundwater monitoring locations); and
- The methodology used for data interpolation should be consistent for the duration of the study.

Groundwater plume mass estimates should therefore be interpreted with caution and may have only order-of-magnitude precision (crcCARE, 2010). Nevertheless, they may provide a useful relative measure of mass in the context of an MNA study. As a minimum, a consistent approach that is representative of the plume area should be used on each occasion to at least allow a relative comparison of mass with time and monitoring well densities should be encouraged to reduce these uncertainties and investigate the sensitivity of the mass estimate to new data.

In terms of estimation of a degradation rate from this data then if an estimate of plume mass is undertaken using the same monitoring well network over a series of time periods then an overall bulk attenuation rate can be calculated by plotting mass versus time (see Figure A5.6). Trends of mass decline or stability (where the attenuation rate matches source mass discharge) should be themselves subject to significance analysis (e.g. Mann-Kendall, or a graphical treatment).

Calculation of mass flux

An alternative mass balance method is to calculate the contaminant mass flux for a given plume (Thornton *et al.*, 2016; Thornton, 2019; Farhat *et al.*, 2006). Contaminant mass flux is the rate at which contaminant mass passes through a defined cross-sectional area perpendicular to the groundwater plume in an aquifer over time. In the context of MNA studies then the calculation and documentation of stable or decreasing mass discharge or flux trends can be a useful secondary line of evidence.

- Mass flux is a rate measurement equal to the contaminant mass moving across a unit area of aquifer perpendicular to the groundwater flow direction. Units are mass/area/time.
- Mass discharge is the total mass of contaminant moving across a control plane (or area of interest) perpendicular to the groundwater flow direction. The area of interest is generally large enough to contain the entire plume. Units are mass/time.

Within the context of the lines of evidence for NA then the assessment of mass flux or mass discharge may be used in two ways:

- Over a series of different transects (control planes) drawn perpendicular to the flow direction of a given plume and the mass flux at each calculated and examined to indicate evidence of mass flux reduction with distance from the source.

- For a single transect and the results repeated over time to indicate reduction in mass flux at a certain control plane over time.

Figure A5.12 is a schematic of a high-resolution site characterisation where three transects have been constructed perpendicular to the groundwater plume to enable detailed characterisation and assessment of mass flux / mass discharge. Figure A5.13 illustrates the calculated mass flux for an example site at four transects at different distances from the source and over three different time intervals.

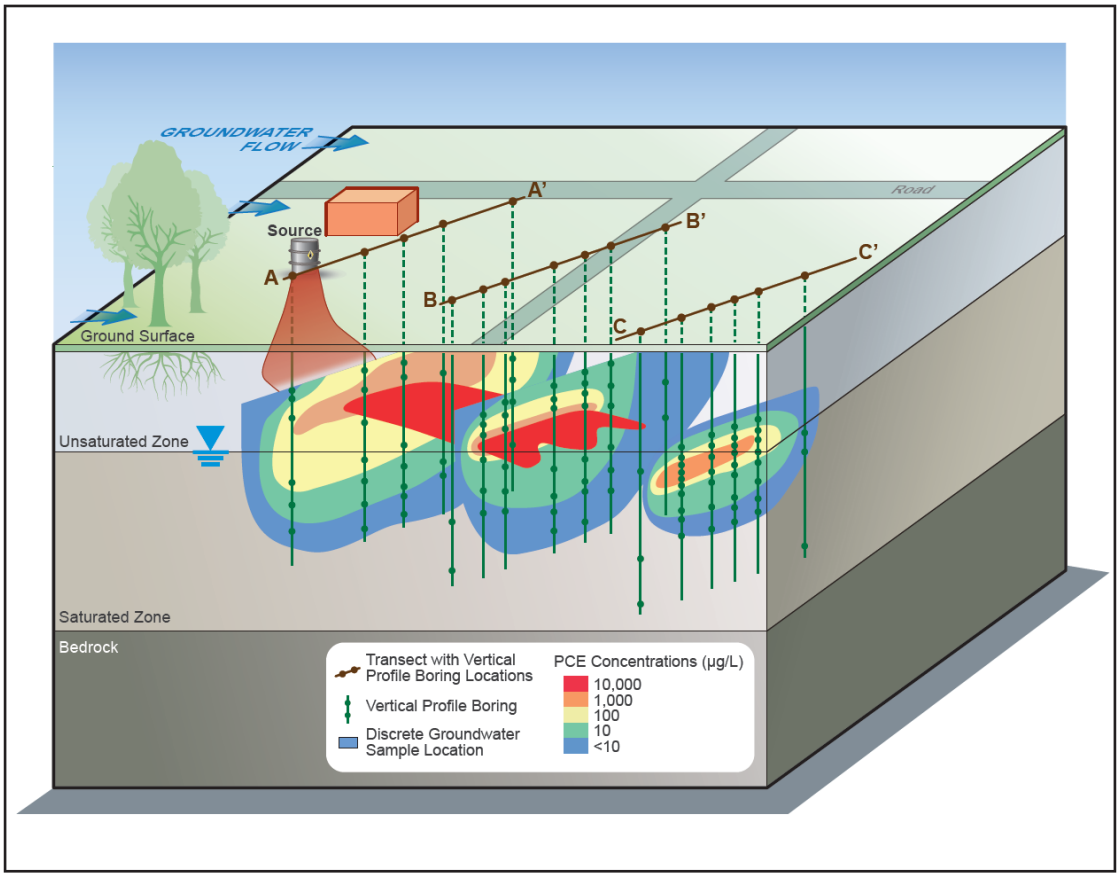


Figure A5.12: Use of multiple well transects (control planes) to measure mass discharge and flux (USEPA, 2021).

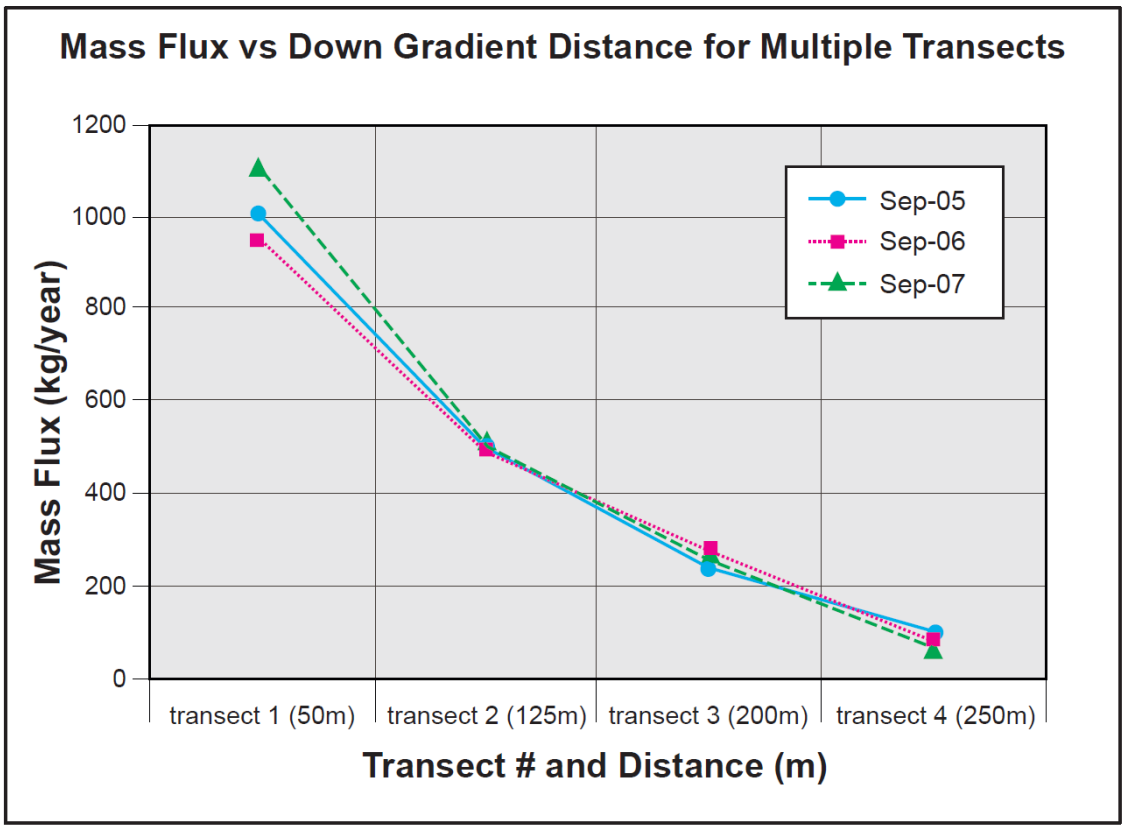


Figure A5.13: Example mass flux with time (crcCARE, 2010).

The Interstate Technology and Regulatory Council (ITRC) in the USA has produced an overview of the concepts, practical use and limitations of mass flux measurement (ITRC, 2010). The document describes a number of methods that are used to measure mass flux and/or mass discharge:

- transects in which individual monitoring points are used to integrate concentration and flow data;
- transects based on contaminant concentration contours, which rely on concentration contour maps developed using groundwater monitoring data;
- well capture/pump test methods, which rely on extracting groundwater and measuring the flow and mass discharge from the wells;
- passive flux meters, which estimate mass flux directly in wells; and
- by using solute transport models that require flow and concentration data as input parameters.

In a mass transect approach the mass of contaminant flowing across a series of lines (control planes) drawn perpendicular (normal) to the flow direction is estimated, as follows:

$$Discharge = \sum (C_{av} W v n D)_{\text{for each depth interval}}$$ Equation A5.5

Where:

Discharge = summation of flux for each depth increment

C_{av} = average contaminant concentration for depth increment [M/L^3]

W = width of plume [L]

v = groundwater velocity [LT^{-1}]

n = kinematic porosity

D = depth increment for each average concentration, or plume thickness [L]

The calculation is repeated for different lines drawn perpendicular to the flow direction. This information can be used to compare the change in contaminant flux with time and distance from the source.

To assist in the calculation of mass flux the Environmental Security Technology Certification Program (ESTCP) of the U.S. Department of Defense (DoD) funded the development of the Microsoft® Excel-based Mass Flux Toolkit (GSI, 2006). The Mass Flux Toolkit is a publicly available software tool with the capability of comparing different mass flux approaches including individual points or contaminant contours, calculating the mass flux from transect data and estimating the uncertainty associated with a calculation.

Uncertainty in mass flux estimates is a key issue in using mass flux as a metric. The Mass Flux Toolkit describes three main sources of uncertainty in a mass flux estimated from transect data (GSI, 2006):

- **Type 1 Uncertainty in the actual concentration, hydraulic conductivity, and hydraulic gradient measurements**. The calculation of mass flux typically relies on an adequate monitoring well network (in terms of locations and vertical density) and ideally would use data from multilevel wells. Data from single long-screened wells are less useful for this technique. The ITRC notes that the greatest sources of error and uncertainty in mass flux or mass discharge estimates include estimates of hydraulic conductivity (K) and contaminant concentrations (ITRC, 2010) and measurements of specific discharge or Darcy velocity, or mass flux *in situ* are recommended.
- **Type 2 Uncertainty in the interpolation scheme**. Different interpolation schemes will result in different mass flux estimates. Some interpolation schemes, such as kriging, provide local estimates of uncertainty.
- **Type 3 Uncertainty associated with unmeasured values**. This type of uncertainty is related to Type 2 uncertainty. However, the uncertainty associated with areas of high mass flux that are missed by the monitoring scheme is difficult to assess. This may be addressed by installing a dense network of monitoring wells which is consistent with the known aquifer heterogeneity (geology/stratigraphy) and spatial variation in preferential flow paths that influence contaminant distribution and transport (Wilson *et al.,* 2004).

A5.3.2 Biodegradation Indicators

Evaluating indicators specific to the biodegradation process is of critical importance when presenting secondary lines of evidence for NA as it is indicative of contaminant destruction (Lelliott and Wealthall, 2004; Rivett and Thornton, 2008; crcCARE, 2010). The microbial processes associated with the degradation of various groups of contaminants are described elsewhere in this document (Section A2.5) but the major electron acceptors and anticipated changes during biodegradation are summarised in Table A5.4.

Table A5.4: Types of biodegradation reactions and preference by energy potential (after Washington State Department of Ecology, 2005).

Type of Microbial Respiration	Electron Acceptor	Metabolic By-Product	Geochemical Indicator Response				Redox Potential Eh (mV@pH 7, 25°C)	
Aerobic (oxidation)	Oxygen	CO_2	O_2	↓	CO_2	↑	+820	Most Preferred
Anaerobic (reduction)	Nitrate (NO_3^-)	N_2	NO_3^-	↓	CO_2	↑	+720	
	Manganese (Mn^{4+})	Mn^{2+}	Mn^{2+}	↑	CO_2	↑	+520	
	Ferric Iron (Fe^{3+})	Ferrous Iron (Fe^{2+})	Fe^{2+}	↑	CO_2	↑	-50	
	Sulfate (SO_4^{2-})	H_2S	SO_4^{2-}	↓	CO_2	↑	-220	
	Carbon Dioxide (CO_2)	Methane (CH_4)	CH_4	↑		↑	-240	Least Preferred

Indicators of biodegradation can be identified graphically (contaminant/daughter product ratios), quantitatively (mass balance and mass flux), or visually (contour/isopleth plots, radial diagrams).

Contaminant ratio plots

Evidence of biodegradation can be obtained by comparing contaminant and breakdown product concentrations or ratios along the flow path. A decrease in CoPC concentration with an associated increase in breakdown product concentration, or an increase in the ratio of breakdown product to parent contaminant concentration, is indicative of biodegradation (Lelliott and Wealthall, 2004; Rivett and Thornton, 2008). Examples of visualisation techniques include:

- plot of contaminant concentrations and breakdown product concentrations with distance (Figure A5.14)
- plot of the ratio of contaminant concentrations with distance (Figure A5.15).

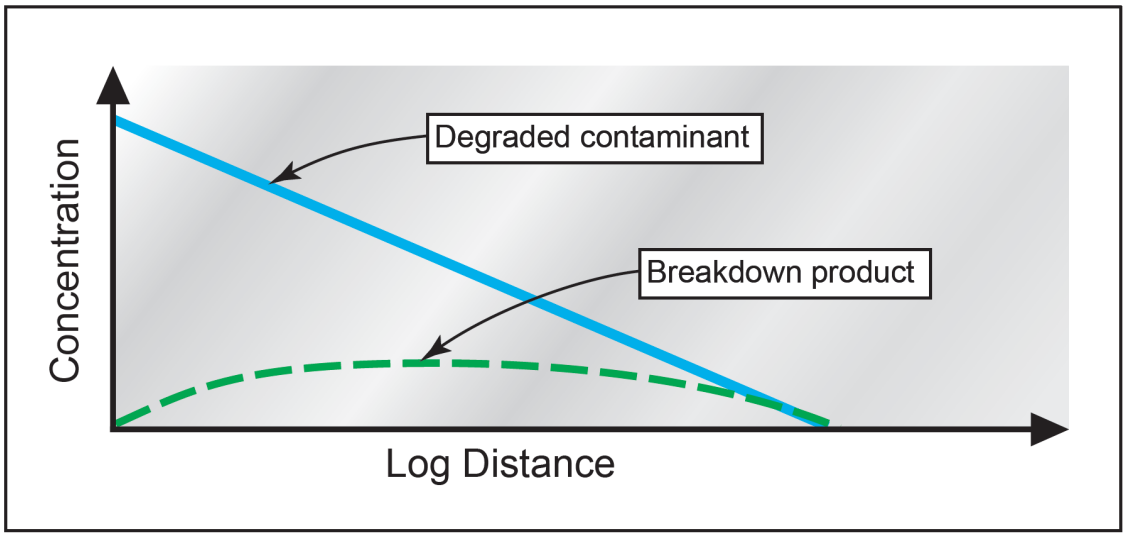


Figure A5.14: Comparison of breakdown products.

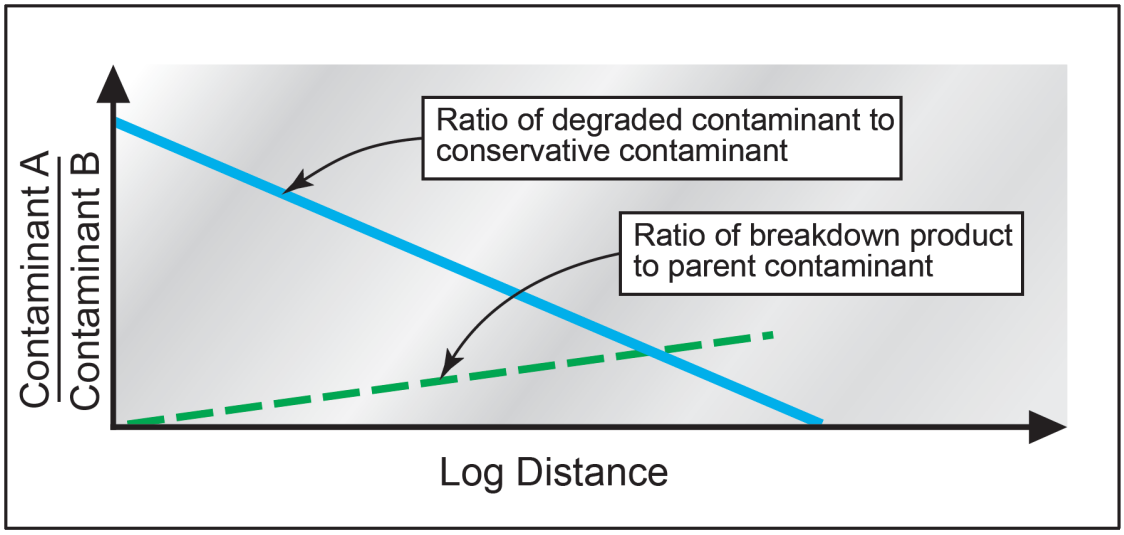


Figure A5.15: Comparison of contaminant ratios.

In assessing contaminant ratios, the following should be taken into account:

- the breakdown products may be present in the original contaminant source (for example, TBA is a breakdown product of MTBE, but this compound is also often a constituent component in petroleum fuels, and TCE may be present with PCE);
- the breakdown product may have been introduced by other contaminant incidents;
- the analytical technique/sampling method may not be appropriate to identify the breakdown product;
- the sorption and volatilisation characteristics of the contaminants may not be identical; and
- the effect of multiple sources or multiple contaminant releases, for example, if the contaminants have a different history of release.

For chlorinated solvents the effectiveness of MNA can include an evaluation of contaminant concentration or mass reduction, particularly as reflected in changing molar concentrations of parent and dechlorination products over time. This includes the following steps (AFCEE, 2004):

Step 1 – Molar Concentration: Calculate the concentration of each compound in mol/L for each compound in the reaction sequence using the equation:

$$\frac{moles_i}{Litre} = \frac{C_i}{MW_i}$$ Equation A5.6

Where:

$moles_i$ = moles of compound i

C_i = concentration of compound i (grams per litre)

MW_i = molecular weight of compound i (grams per mole)

Step 2 – Total Molar Concentration: Calculate the total concentration in moles per litre by summing the concentrations of each compound in the reaction sequence.

To illustrate, consider the chlorinated ethenes with PCE as the parent compound:

$$\sum \frac{moles_{ethenes}}{litre} = \frac{C_{PCE}}{MW_{PCE}} + \frac{C_{TCE}}{MW_{TCE}} + \frac{C_{DCE}}{MW_{DCE}} + \frac{C_{VC}}{MW_{VC}} + \frac{C_{Ethene}}{MW_{Ethene}}$$

Equation A5.7

Where :

$$\sum \frac{moles_{ethenes}}{litre} = total\ chlorinated\ ethenes(mol/L)$$

Step 3 – Molar Fractions: Calculate the molar fraction (ratio) for each compound.

For illustration, consider PCE. This calculation must also be completed for TCE, DCE, VC, and ethene.

$$MF_{PCE} = \frac{\frac{moles_{PCE}}{litre}}{\sum \frac{moles_{ethenes}}{litre}}$$ Equation A5.8

Where:

MF_{PCE} = molar fraction of PCE (unitless)

An example of a visualisation created using the above is shown in Figure A5.16.

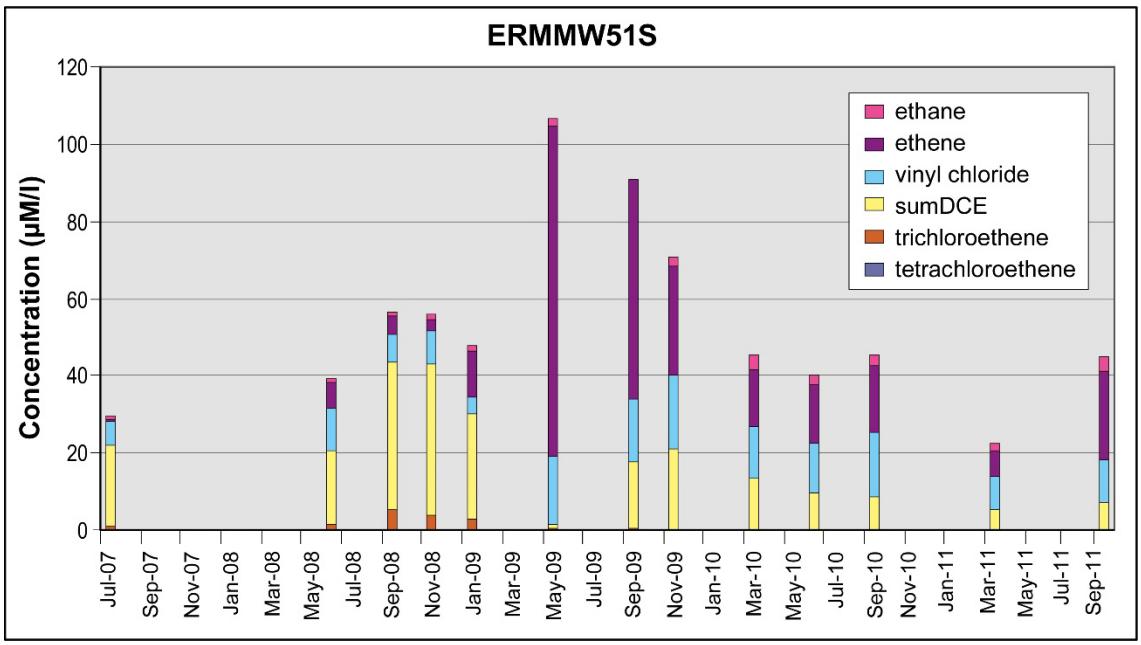


Figure A5.16: Change in molar concentration over time in a chlorinated solvent-impacted monitoring well (Source: ERM).

Mass balance mass flux methods

The mass balance, mass flux method, as detailed in Section A5.3.1, can also be used to monitor the change in mass of CoPC breakdown products. An inverse relationship is expected between concentration changes for CoPC and associated breakdown products (Lelliott and Wealthall, 2004) as indicated in Figure A5.17 for chlorinated solvents and Figure A5.18 for phenols.

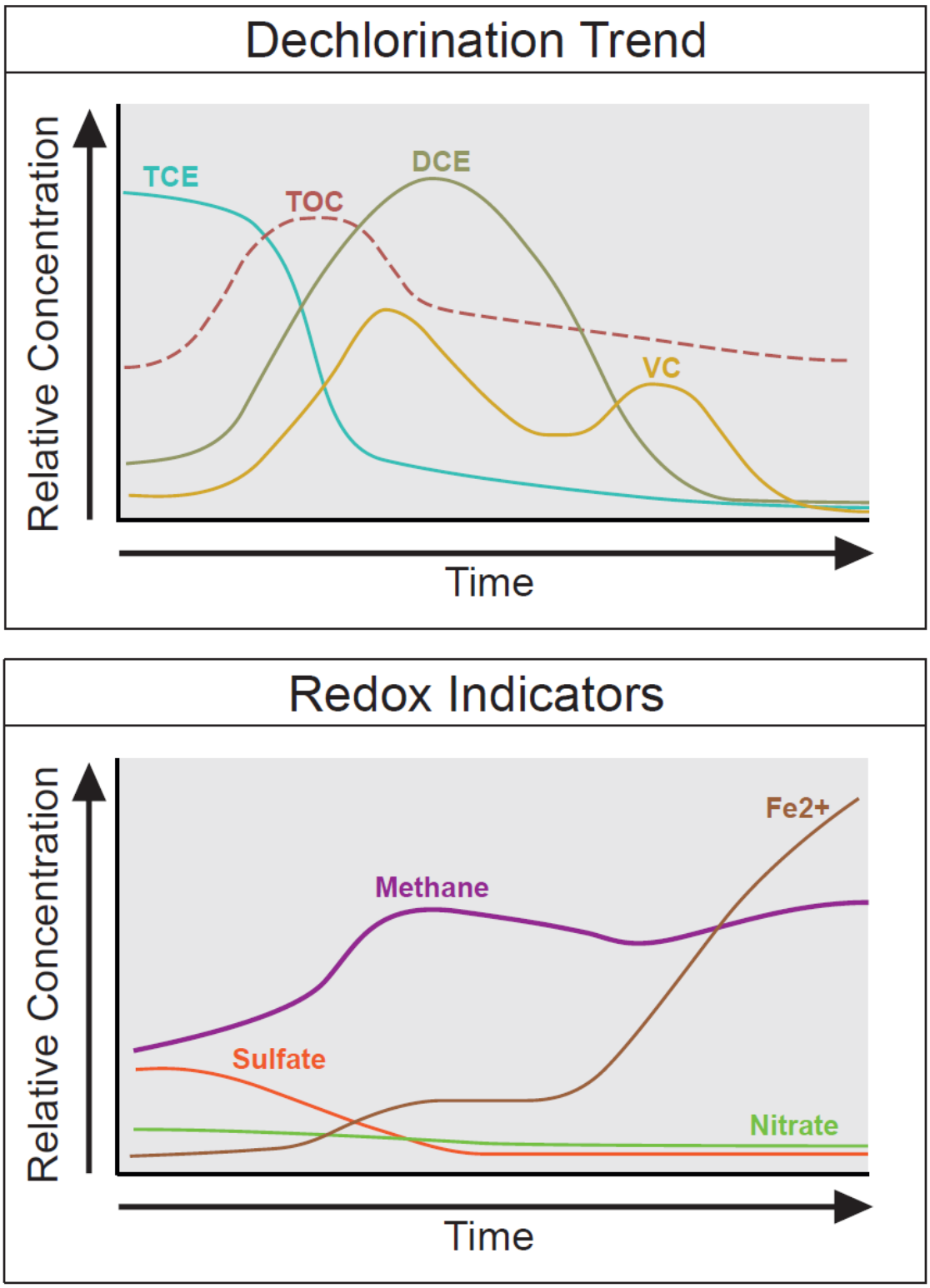


Figure A5.17: Relationship between parent compound (TCE), breakdown products (DCE, VC) and geochemistry over time in a chlorinated solvent-impacted monitoring well (Source: ERM).

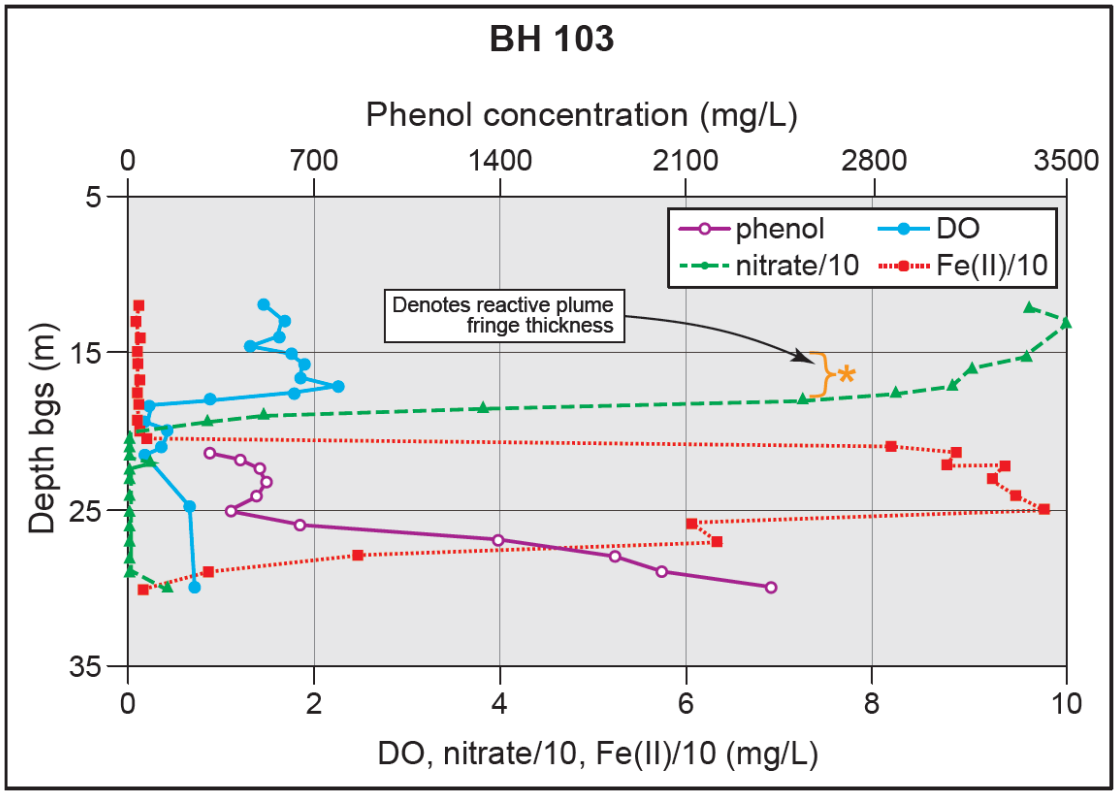


Figure A5.18: Relationship between phenol concentration and electron acceptors over discrete high-resolution vertical intervals within a multi-level monitoring well (Wilson *et al.*, 2005).

Contour/isopleth plots

Similarly biodegradation can also be assessed visually using contour/isopleth plots for breakdown products, electron acceptors/donors, and hydrochemical indicators. Contour plots can be used to indicate the areal extent of indicators, or the vertical distribution and should be for different time periods to identify temporal changes in indicator concentrations (Lelliott and Wealthall, 2004; Rivett and Thornton, 2008).

Contour plots of CoPC breakdown products provide visual evidence of where biodegradation is occurring, and there should be an inverse relationship between CoPC and breakdown product concentration (Figure A5.19).

It is important to identify if the breakdown product is present in the source zone, or introduced as a separate incident (multiple sources or contaminant releases), as this could give a false indication of biodegradation (e.g. road gritting in winter can lead to erroneously high chloride that can mislead evaluations of chlorinated solvent degradation). Multiple lines of evidence are required in this case.

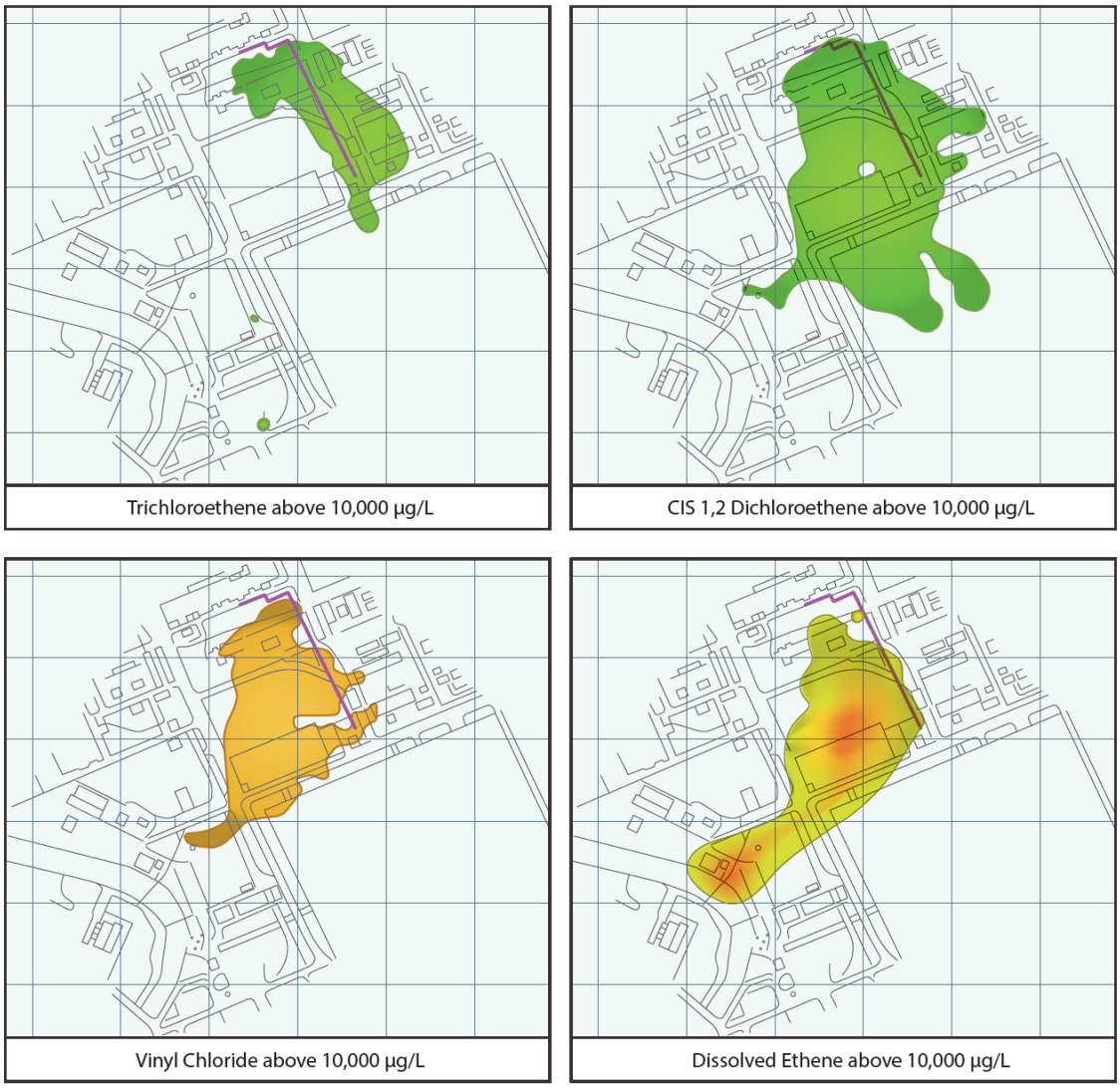


Figure A5.19: Spatial relationship between parent compound (TCE) and breakdown products (DCE, VC and ethene) in a chlorinated solvent plume (Source: ERM).

Radial Diagrams

A radial diagram visualisation approach allows simultaneous comparison of spatial and temporal trends for multiple chemicals on one map (Lelliott and Wealthall, 2004). In this approach a radial diagram is constructed with each of the axes assigned to either the primary source of contamination, degradation products or electron acceptors (Carey *et al.,* 2003). Concentrations are then plotted on these axes as shown in Figures A5.20 and A5.21. Variations of radial diagrams include pie charts or bar charts where segments are defined according to electron acceptor concentrations.

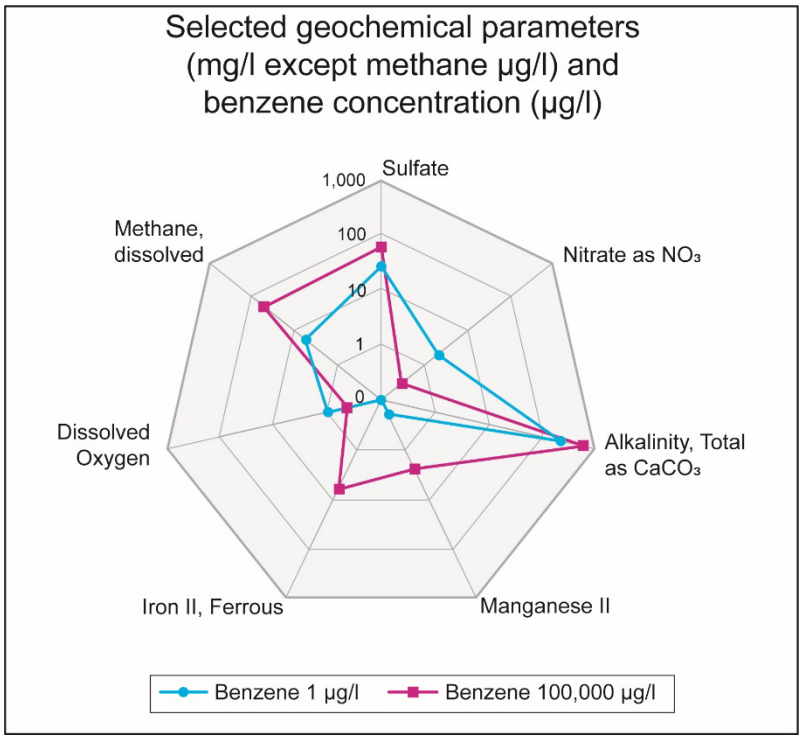


Figure A5.20: Relationship between different electron acceptor concentrations in two monitoring wells with high (>100,000 µg/l) and low (1 µg/l) benzene contamination, indicating typical changes expected as a result of microbial activity (Source: ERM).

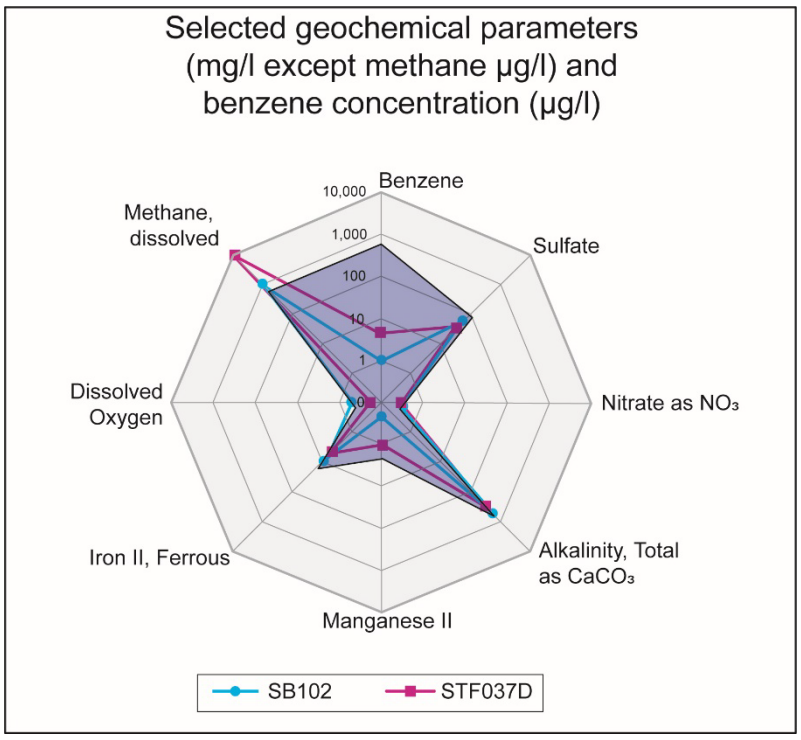


Figure A5.21: Spatial relationship between benzene and different electron acceptor concentrations in source monitoring well (shaded area) and downgradient monitoring wells (Source: ERM).

Groundwater at the source or upgradient can be used to provide the initial baseline graphical shape and this can then be compared spatially with water chemistry from other parts of the site, or alternatively in the same location over time. Changes in the shape of the graphic provide visual evidence of changes in concentration of either the contaminants or electron acceptors.

Radial diagrams can be produced via proprietary software Visual Bio or can be produced in Excel.

Figure A5.22 presents an example of a Visual Bio radial diagram showing the CoPC and sequential breakdown products for TCE and how the relative concentrations change along the contaminant plume. The outer data series (indicated by the blue line) represents the concentration levels for each of these contaminants as they are measured at the source of contamination. The inner data series (indicated by the yellow shading) represents the concentrations of these contaminants measured at the monitoring wells located downgradient from the contaminant source. This diagram shows decreasing concentrations of contaminants downgradient of the source (primary lines of evidence), and the increased concentration of breakdown products (e.g. VC and chloride) provide evidence of intrinsic biodegradation (secondary lines of evidence) of the CoPC (Lelliott and Wealthall, 2004).

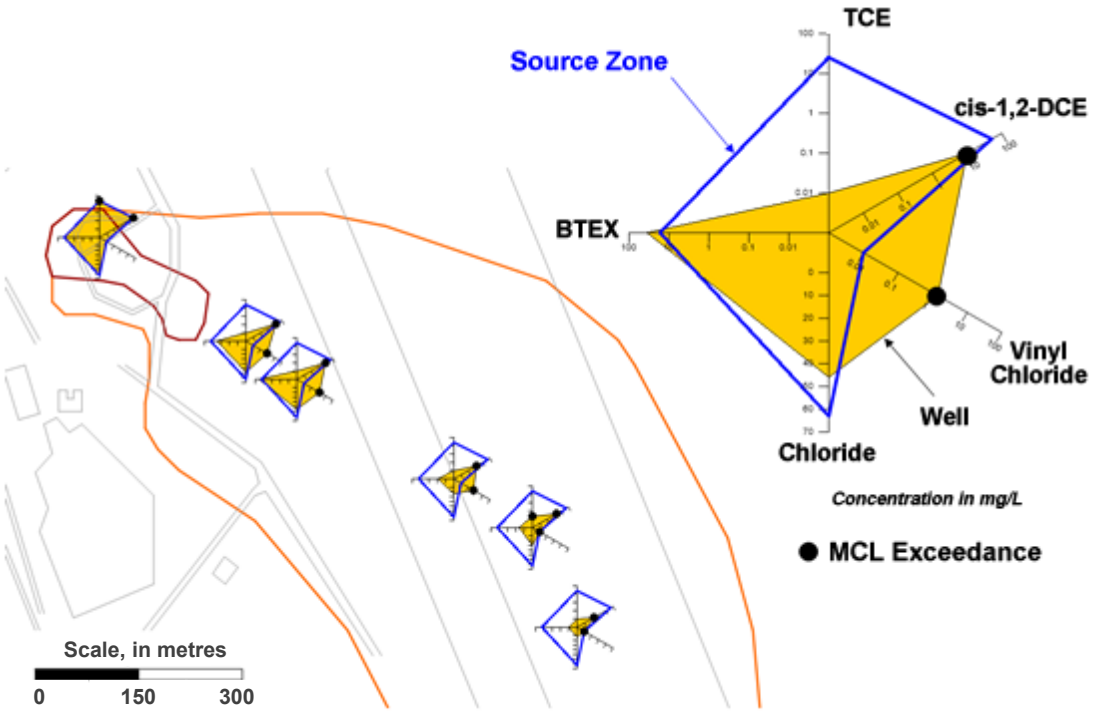


Figure A5.22: Example of output from Visual Bio Software (Carey *et al.*, 2003). © 2003 John Wiley & Sons, Inc.

The use of radial diagrams allows clear comparison of multiple related chemical parameters (i.e. parent and breakdown products, sequential redox indicators) at individual monitoring wells, and also between monitoring wells. Radial diagrams also do not involve any data interpolation and are based on real chemical concentrations (Lelliott and Wealthall, 2004).

A5.4 Tertiary Lines of Evidence

Tertiary lines of evidence typically use data from laboratory microbiological testing to show that indigenous bacteria are capable of degrading site contaminants (Rivett and Thornton, 2008; Thornton, 2019). Conventionally this would have involved microbial testing such as conducting plate counts, enrichment cultures and microcosm studies. In the past it was anticipated that this line of evidence would only be required when primary and secondary lines of evidence are inconclusive as it can be both costly, time consuming and sometimes inconclusive. However, with the emergence of molecular biological tools (MBTs) and compound specific isotope analysis (CSIA) on a commercially-available basis there have been significant advances in the ability to provide unequivocal evidence of contaminant biodegradation and these tools are being used on a much more frequent basis to support MNA studies (Thornton *et al.*, 2016).

Recognising the importance of these techniques, a separate dedicated appendix describing the key features of each is provided elsewhere in this document (Appendix 8 for CSIA and Appendix 9 for MBTs). In summary MBTs are defined as techniques that target biomarkers (specific nucleic acid sequences, peptides, proteins or lipids) to provide information about organisms and processes relevant to the assessment or remediation of contaminants (NJDEP, 2012) and CSIA is the measurement of the isotope fractionation (typically, the stable isotope ratios of carbon, hydrogen or chlorine) of individual volatile and semi-volatile compounds extracted from complex environmental mixtures (Thornton *et al.*, 2016; USEPA, 2008). Data visualisation methods associated with the results from both MBTs and CSIA typically include means of spatial or temporal analysis and correlation of results with either changes in contaminant chemistry or electron acceptors as described above.

Coupled with increasing recognition and use of these techniques has been the development of additional guidance and tools with which to quantify the potential for NA. For chlorinated solvents one such tool is called BioPIC (Biological Pathway Identification Criteria) and was developed by Lebrón *et al*. (2015) as a quantitative framework to evaluate whether MNA is an appropriate remedy based on site-specific conditions. BioPIC consists of a decision flow chart that uses the quantitative relationships between biotic and abiotic parameters that contribute to the detoxification of chlorinated ethenes and determine degradation rates. It allows the user to determine if degradation is occurring and, if it is, to deduce the relevant degradation pathway(s) based on the assessment of specific analytical parameters. While the document is focused on the demonstration of MNA in the context of the US regulatory environment it does contain a number of tools that document the relationship between several biotic and abiotic indicators and calculated degradation rates (Figure A5.23). These can assist in answering the question whether the observed degradation can be explained by the particular indicator as evidence of NA and are relevant in the UK or internationally. These indicators include:

- Quantification of reductive dechlorination genes to estimate whether biostimulation is necessary;
- Measurement of the abundance of *Dehalococcoides sp.* in the subsurface to help predict the first-order decay rate;

- Evaluation of stable isotope analysis to look for evidence of fractionation that is indicative of degradation; and
- Evaluation of the role of iron sulfide in abiotic reduction.

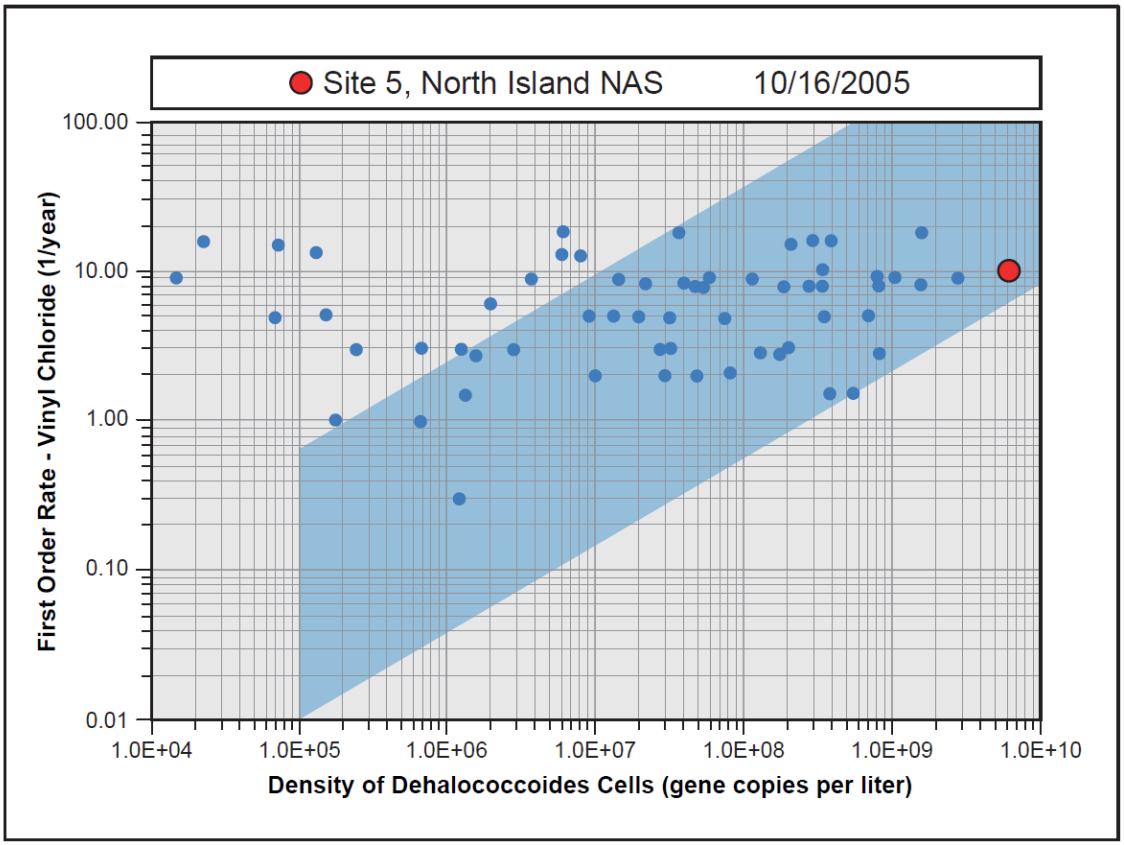


Figure A5.23: Example of output from relationship between the density of *dehalococcoides* and observed first-order decay rates of VC contained in BioPIC tool. If data plot within the blue zone outlined, then the abundance of *dehalococcoides* in groundwater can explain the *in situ* rate of VC degradation (Lebrón *et al*., 2015).

A5.5 Optional Lines of Evidence

A5.5.1 Demonstration of Assimilative Capacity of an Aquifer

An electron mass balance calculation can be used to give an indication of the capacity of an aquifer to degrade contaminants (its assimilative capacity). The approach relies on the measurement of changes in groundwater chemistry at a site together with a stoichiometric relationship describing the amount of contaminant degraded through oxidation/ reduction reactions.

The amount of a contaminant, such as benzene, that can be theoretically degraded by an electron accepting process can be estimated from Equation A5.9 as follows:

$$BC = \sum \frac{C_B - C_P}{F} = \qquad \text{Equation A5.9}$$

Where:

BC = biodegradation capacity (mg/l)

C_B = average background concentration of electron acceptor or metabolic by-product (mg/l)

C_P = lowest measured electron acceptor or metabolic by-product concentration within plume (mg/l)

F = contaminant utilisation factor (mg/mg)

$\sum$ = sum of electron acceptor and metabolic by-products that contribute to degradation

The biodegradation capacity (BC) is the equivalent amount of contaminant that the electron acceptors can assimilate or degrade based on the observed electron-acceptor capacity of the aquifer. Note that BC here is based on observations of electron acceptors consumed (obtained from monitoring data) and may be much less than the total theoretical BC of the aquifer, based on the unused mass of electron acceptors (e.g. metal oxide content of aquifer material). This will be a function of:

- Groundwater flow beneath the contaminant source; and
- Recharge/infiltration over the contaminant source area;

The total biodegradation capacity (TBC) of the system can be estimated as:

$$TBC = 1000 \times Q \times BC \qquad \text{Equation A5.10}$$

Where:

TBC = total biodegradation capacity (mg/d)

Q = groundwater flow through plume (m^3/d)

BC = biodegradation capacity (mg/l)

This calculation can be used to determine whether the BC of the system is sufficient to have degraded the mass of contaminant. The method can also be used to indicate the relative importance of different electron acceptor/metabolic by-products to degradation. The method should be used only as a qualitative tool in assessing the degradation process, due to uncertainties regarding the cause of the oxidation/reduction reaction though may be supplemented/supported by other measurements including CSIA and MBTs. In some circumstances reducing conditions may be natural and for other sites more than one contaminant may be competing for the electron acceptors.

Examples of electron balance calculations are given in AFCEE (2004) and Wiedemeier *et al*. (1999). A more sophisticated electron balance methodology is described for plume-scale mass balances in Thornton *et al.* (1998) and Thornton *et al.* (2001), which shows that dispersion/mixing at the plume margins can be a significant source of soluble electron acceptors (e.g. dissolved oxygen, nitrate and sulfate) for contaminant biodegradation.

Electron balance calculations are included in a number of fate and transport models, for example, CoronaScreen, RT3D and NAS (see Table A7.1, Appendix 7). They indicate the capacity of all terminal electron accepting processes to oxidise single or mixed

contaminant plumes, integrated with flow and transport processes, and may be regarded as semi-quantitative or quantitative methods compared to Equation A5.10.

An example of oxidation/reduction processes is given in Table A5.5 for the degradation of benzene. This table also gives the mass of benzene degraded per unit mass of electron acceptor consumed and metabolic by-product produced.

Table A5.5: Electron acceptors and metabolic by-products involved in the degradation of benzene.

Process	Acceptor or metabolic by-product	Reaction	Mass of benzene degraded per unit mass of electron acceptor (-)	Mass of benzene degraded per unit mass of metabolic by-product produced (-)
Oxidation	Oxygen	$7.5O_2+C_6H_6 \rightarrow 6CO_2+3H_20$	0.33	-
Denitrification	Nitrate	$6NO_3+6H^++C_6H_6 \rightarrow 6CO_2+6H_20+3N_2$	0.21	-
Sulfate reduction	Sulfate	$7.5H^++3.75SO_4^{2-}+ C_6H_6 \rightarrow 6CO_2+3.75H_2S+3H_20$	0.22	-
Manganese reduction	Manganese	$30H^++15MnO_2+C_6H_6 \rightarrow 6CO_2+15Mn^{2+}+18H_20$	-*	0.094
Iron reduction	Iron	$60H^++30Fe(OH)_3+ C_6H_6 \rightarrow 6CO_2+30Fe^{2+}+78H_20$	-*	0.046
Methanogenesis	Methane	$4.5H_2O+C_6H_6 \rightarrow 2.25CO_2+3.75CH_4$	-	1.3

* The masses of MnO_2 and $Fe(OH)_3$ (solid phase electron acceptors) consumed during anaerobic benzene biodegradation are not shown. In practice, these metal oxide fractions are not measured, whereas the Mn^{2+} and Fe^{2+} by-products of these redox processes are measured in groundwater to estimate the aquifer BC, based on the appropriate utilisation factors of 0.094 and 0.046, respectively.

Similar reactions for toluene, xylene, ethylbenzene and chlorinated solvents reflecting the stoichiometry of the degradation of these compounds are given in AFCEE (2004) and Wiedemeier *et al*. (1999).

Example – Degradation of BTEX

An example of the calculation of the BC for a contaminant plume (with BTEX compounds as the main contaminants of concern) is given in Table A5.6. In this example the measured difference in the concentration of electron acceptors and metabolic by-products would, in theory, be equivalent to the degradation of 13.6 mg/l of BTEX. For a groundwater throughput of 100 m^3/d, then a total of 1.36 kg/d of BTEX could be degraded. This amount can be compared with the volume of contaminant lost or the calculated rate of dissolution of BTEX from a NAPL source.

Table A5.6: Example calculation (degradation of BTEX)[1].

Electron acceptor/ metabolic by-product	Upgradient concentration (mg/l)	Plume concentration (mg/l)	Difference (mg/l)	Biodegradation capacity[2] (mg/l)
Oxygen	5.2	0.1	5.1	1.63
Nitrate	4.3	0.1	4.2	0.84
Sulfate	34.0	8.0	26	5.46
Manganese	0.01	1.2	1.2	0.11
Iron	0.01	2.4	2.4	0.11
Alkalinity	210	240	30	3.9
Methane	0.01	1.1	1.1	1.4
Biodegradation capacity or quantity of BTEX that could theoretically be degraded				13.5

[1] Utilisation factors (UF) for the BTEX group of chemicals are slightly different than those for benzene alone (i.e. those shown in Table A5.5). Respective values of UF for the BTEX group have been used to correct the data in Table A5.6.

[2] Biodegradation capacity = difference in concentration of electron acceptors up-hydraulic gradient and within the plume divided by the Utilisation Factor, (see Table A5.7 below)

Utilisation factors for the electron acceptors and metabolic by-products that are involved in the degradation of BTEX are given in Table A5.7.

Table A5.7: Utilisation factors.

Electron acceptor/ metabolic by-product	Utilisation factor[1]
Oxygen	3.14
Nitrate	4.87
Sulfate	4.76
Manganese	6.67
Iron	21.8
Alkalinity	7.69
Methane	0.78

[1] mass of electron acceptor consumed per unit mass of BTEX degraded

A5.5.2 Estimation of the Source and/or Plume Depletion and Longevity

The effectiveness of NA could be further demonstrated by rates of source and/or plume depletion and longevity:

a) Predicting a concentration decline (or other performance metric - source mass, source mass discharge, plume mass, plume area, plume length etc. depending on what the remedial objectives are) versus time to meet a specific concentration (or other) objective. For example, if concentration is a relevant metric then the Point Attenuation Rate (K_{point}, time per year) summarised in Table A5.3 can be extrapolated to predict when concentrations will meet a remedial objective. This can be done applying an upper confidence limit on the data to add some acknowledgement to data variability and uncertainty in predictions.
b) Estimating via a model - a number of models and their use in MNA studies are described within Appendix 7 (use of Bioscreen, BIOCHLOR, RemChlor, REMChlor MD, NAS etc.) but there are other models that can provide insights to MNA duration, for example, SourceDK (Farhat et al., 2011) and the Matrix Diffusion Toolkit (Farhat *et al*, 2012).

Appendix 6: Implementation – Performance Monitoring and Verification

A6.1 Introduction

Implementation is the fourth step in the MNA process (Figure 1). Implementing MNA involves continuation of groundwater monitoring, termed “performance monitoring”, to verify remediation objectives have or will soon be achieved. The purpose of performance monitoring is to demonstrate that NA continues to be an effective remediation strategy that is protective of identified receptors.

The objectives of performance monitoring are to provide sufficient, reliable data to:

- Demonstrate that there is no impact or imminent risk of impact to downgradient receptors;
- Confirm compliance with remediation criteria;
- Demonstrate that NA is occurring according to expectations;
- Provide a basis to close out MNA; and
- Identify change, especially reduction, in the effectiveness of MNA due to change in conditions (e.g. modified groundwater flow direction, increased plume mass discharge etc.) and provide a basis for effecting the Contingency Plan, if required.

Figure A6.1 summarises the main steps in performance monitoring and verification of MNA. The feasibility of MNA as a groundwater remediation strategy will depend on whether the Monitoring Plan required to provide data to verify the remedial objectives have been achieved can be implemented.

These objectives can be demonstrated through extension of the CSM for flow and transport of the contaminants, established during Steps 2 and 3 of the MNA process (Figure 1).

A6.2 MNA Performance Monitoring Strategy

The performance monitoring strategy, that includes a monitoring plan and contingency plan, is site-specific and based on the conceptual model for MNA. The strategy, monitoring and contingency plans should be agreed with the regulator and other key stakeholders. The basic elements of the strategy should consider the steps described in the following section (A6.2.1).

A6.2.1 Remediation Objectives and Criteria

Remediation objectives that are protective of identified receptors should be set on a site-specific basis to decide: (i) when monitoring can cease and (ii) when remediation alternatives to MNA should be considered. Remediation criteria are required to support either proposition, based on the data collected during performance monitoring.

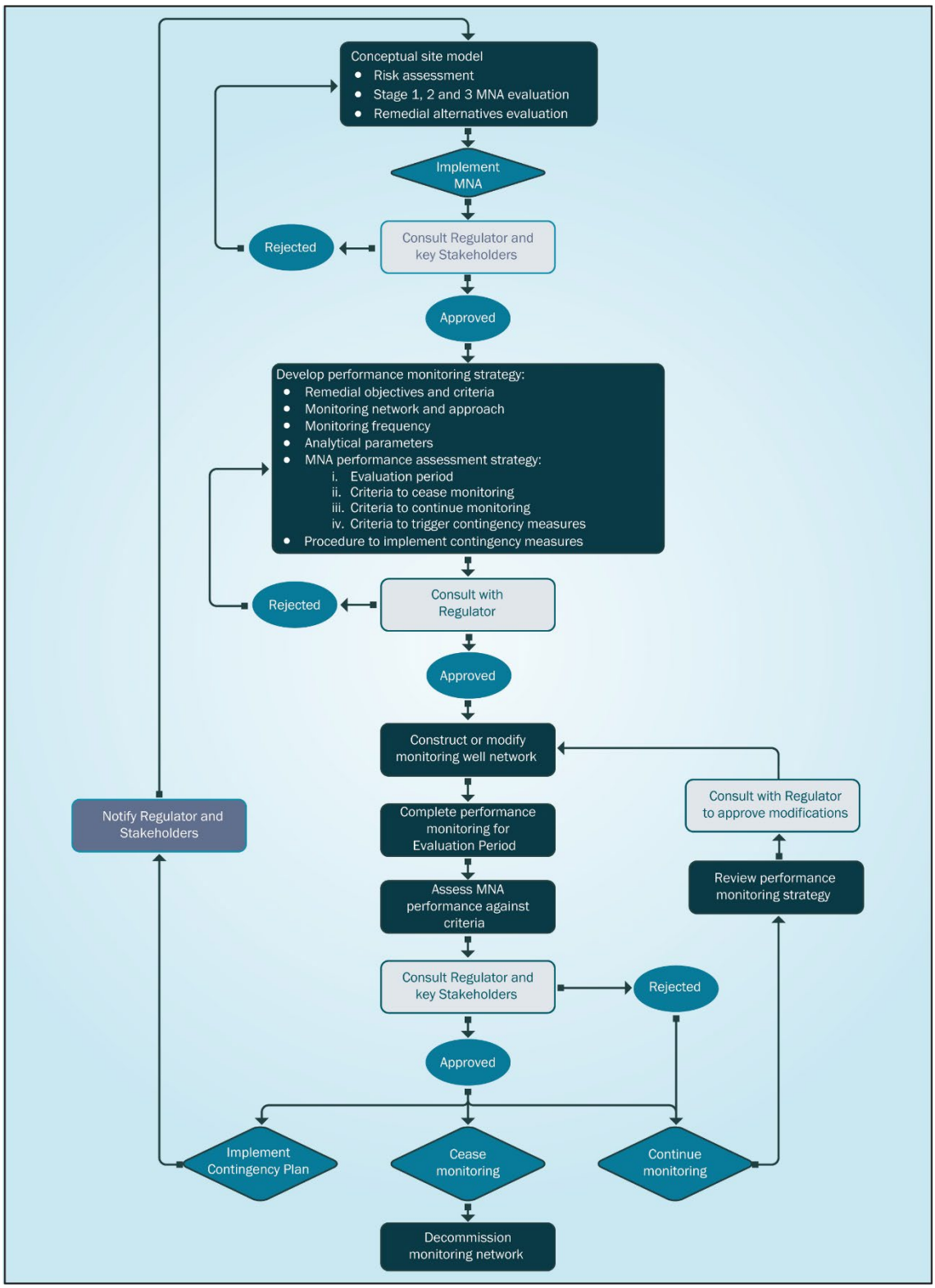


Figure A6.1: MNA performance monitoring and verification.

The principal objective of implementing an MNA strategy is demonstration of the long-term protection of downgradient receptors due to NA. NA processes are taken into account in risk-based assessments for groundwater and surface water at Level 3 and Level 4 of the Environment Agency's Remedial Targets Methodology (Environment Agency, 2006). Risk-based target concentrations developed from Level 3 or Level 4

assessments performed according to this methodology may be applied as remediation criteria for MNA, where:

- The risk assessment is representative of the critical NA processes evident in site-specific data demonstrating MNA viability and associated uncertainties in these data;
- Downgradient points of compliance are appropriately established with consideration to the contaminant, pathway (aquifer) and sensitivity or use of the receptor (e.g. statutorily protected wetlands, water resources).

In setting remediation criteria for MNA demonstrating absence of risk to receptors, the performance monitoring strategy should also define:

- The location(s) at which data indicating compliance with the remediation criteria can be physically measured over time, for example monitoring well(s), which may be receptor, contaminant and/or pathway-specific;
- How and when compliance with the remediation criteria will be assessed. Statistical methods incorporating a pre-determined review period or frequency will typically be required to demonstrate compliance has been achieved or will be achieved in future, to an adequate level of confidence.

In some cases, contaminant concentrations may already be below remediation criteria and performance monitoring is required for confirmation purposes only. Performance monitoring data demonstrating ongoing compliance with risk-based target concentrations may support decisions to reduce monitoring frequency or cease monitoring.

A secondary objective of the MNA strategy is to indicate when NA is no longer sufficiently effective and contingency measures may be required. Trigger criteria should also be established to provide advance warning that MNA is not performing as expected and indicate when the Contingency Plan should be implemented.

Trigger criteria may be based around the following conditions potentially indicating underperformance of MNA:

- Arrival of contaminants, including contaminative degradation products, at the receptor(s) or in sentinel monitoring points;
- Adverse change in observed rates or geochemical conditions of key NA processes (e.g. biodegradation);
- Reversal of mass and/or concentration trends in source and/or performance monitoring wells;
- Contaminant mass or concentrations not decreasing at a sufficient rate to meet remediation objectives within the desired timeframe, and/or;
- Other non-technical circumstances potentially triggering the need for contingency measures, such as insolvency of the operator.

A6.2.2 MNA Monitoring Plan

Monitoring Network and Approach

The location, number and type of monitoring points will depend upon the complexity, spatial and temporal variability of the groundwater flow regime, the size and stability of the plume, relative levels of contamination and the location and sensitivity of receptors.

Monitoring points will comprise mostly groundwater monitoring wells potentially with monitoring in abstraction wells, springs and/or surface waters.

A typical performance monitoring network (Figure A6.2) will include:

- Upgradient well(s) to determine changes in background water quality and enable assessments of MNA performance relative to background conditions;
- Well(s)/well transect(s) immediately downgradient of the source zone(s) to monitor changes in source mass discharge with time;
- Well(s)/well transect(s) located within the plume(s) to monitor behaviour and dynamics;
- Well(s) delineating the plume fringes to monitor changes in plume geometry;
- Sentinel well(s) located between the plume(s) and the identified receptor, to provide early warning of imminent impact(s) at the receptor;
- Monitoring points at the receptor, including abstraction wells, springs and/or surface waters.

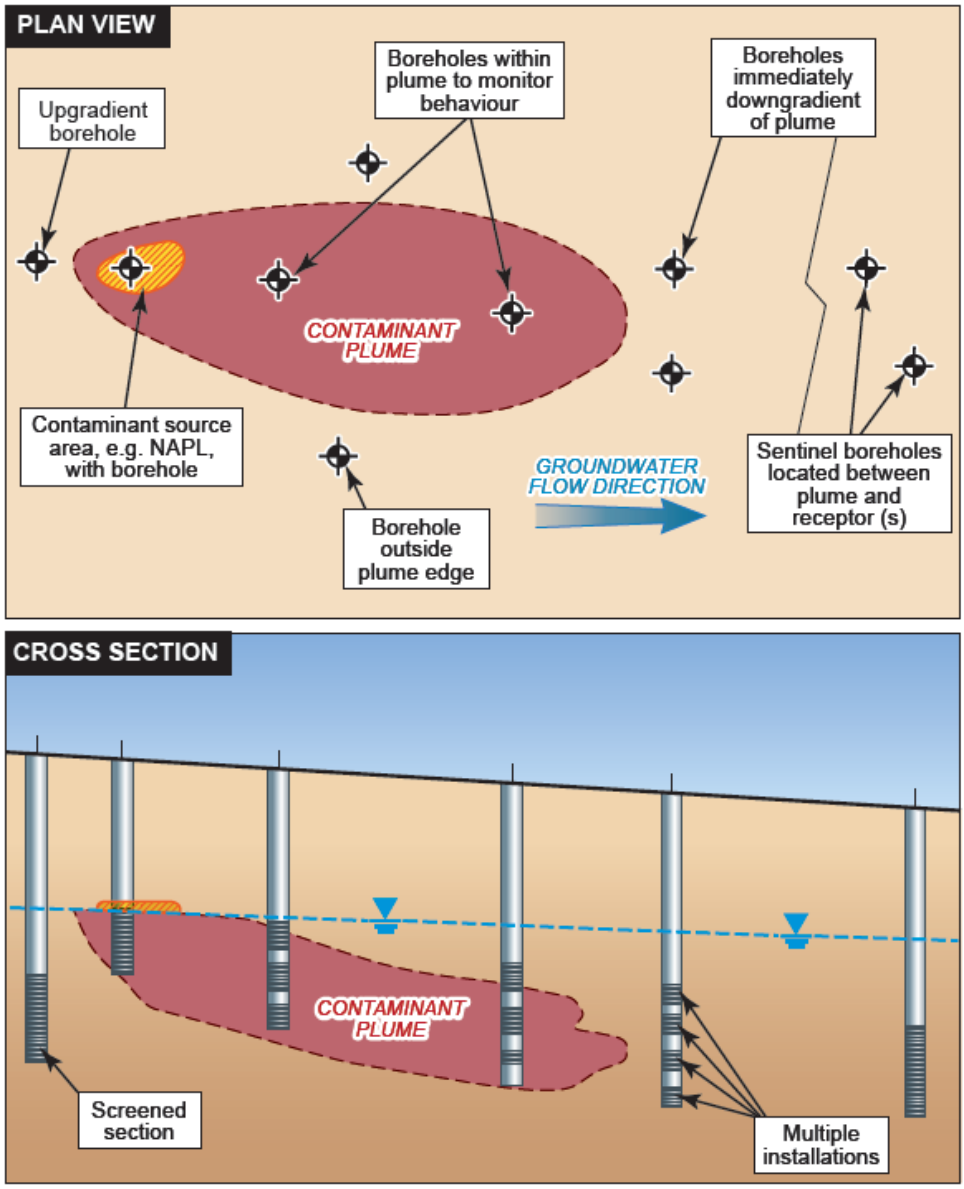


Figure A6.2: Schematic location of performance monitoring well network around a plume.

The configuration of performance monitoring points will be dependent on the distribution and behaviours of the source(s) and plume(s) indicated by the conceptual model for MNA and the location and type of receptor (e.g. source protection zone). Monitoring points used for these earlier stages of MNA may therefore be reused for performance monitoring.

At most MNA sites, monitoring wells tend to be added sequentially during characterisation in a process that can take years to decades. The result can be a well network with tens to hundreds of wells that may have prolific redundancies in spatial coverage. Depending on the overall stability of the plumes being monitored, there can be substantial opportunities to streamline both the monitoring network[1] as well as the frequency of monitoring. Geospatial and spatio-temporal modelling techniques (e.g. MAROS [Aziz *et al.,* 2000]; Geostatistical Temporal-Spatial (GTS) algorithm [Cameron, 2004]; McLean, 2018; Torres, 2019) provide means of assessing the optimal performance monitoring network, utilising the considerable volume of data typically collected to demonstrate MNA viability.

The monitoring approach should aim to collect data that are both representative and comparable with preceding stages of MNA evaluation. Groundwater sampling technologies are well-established and described in existing guidance (e.g. CL:AIRE, 2008). MNA performance monitoring may be required over a period of months to years. Repeatable groundwater sampling procedures, that are effective at mitigating sources of short-term variability in monitoring data (e.g. Kulkarni *et al*., 2015), are required to manage noise in monitoring data that may confound evaluation of long-term MNA performance.

Monitoring Frequency

The monitoring frequency should be designed to detect changes in site parameters that indicate the potential for MNA to meet remedial objectives, whilst ensuring that the receptor(s) remain protected. The monitoring frequency should therefore be determined on a site-specific basis, considering observed plume dynamics, such as velocity, stability, concentration trends and rate of change, as well as the magnitude and consequences of risks being managed. The monitoring frequency should be agreed in consultation with the regulator in advance of implementing MNA.

At many sites, changes in contaminant and biogeochemical systems can be gradual and take several years to manifest. While there is natural variability in long-term groundwater monitoring data (Newell *et al*., 2002), contributions to the observed variability from other sources[2] can be large (60 to 70% [McHugh *et al*., 2011]), that mask long-term temporal concentration trends and limit the ability to understand the performance of MNA (Figure A6.3). Recent research shows that frequent monitoring (<1 year) serves mostly to characterise this time-independent variability rather than the long-term time-dependent trend of interest (McHugh *et al*., 2015; Kulkarni *et al*., 2015). The optimal

[1] Monitoring wells that are not required for performance monitoring may be decommissioned if they are unlikely to be reinstated in the event that contingency measures are triggered.

[2] Time-independent sources of variability in contaminant concentrations include, but are not limited to, aquifer and monitoring well dynamics, sample collection and handling procedures, and sample analysis (McHugh *et al*., 2015).

MNA performance monitoring frequency may therefore be longer (typically >1 year for most systems) compared to the frequency of data collection previously used to demonstrate that MNA is a viable risk management strategy (typically <<1 year).

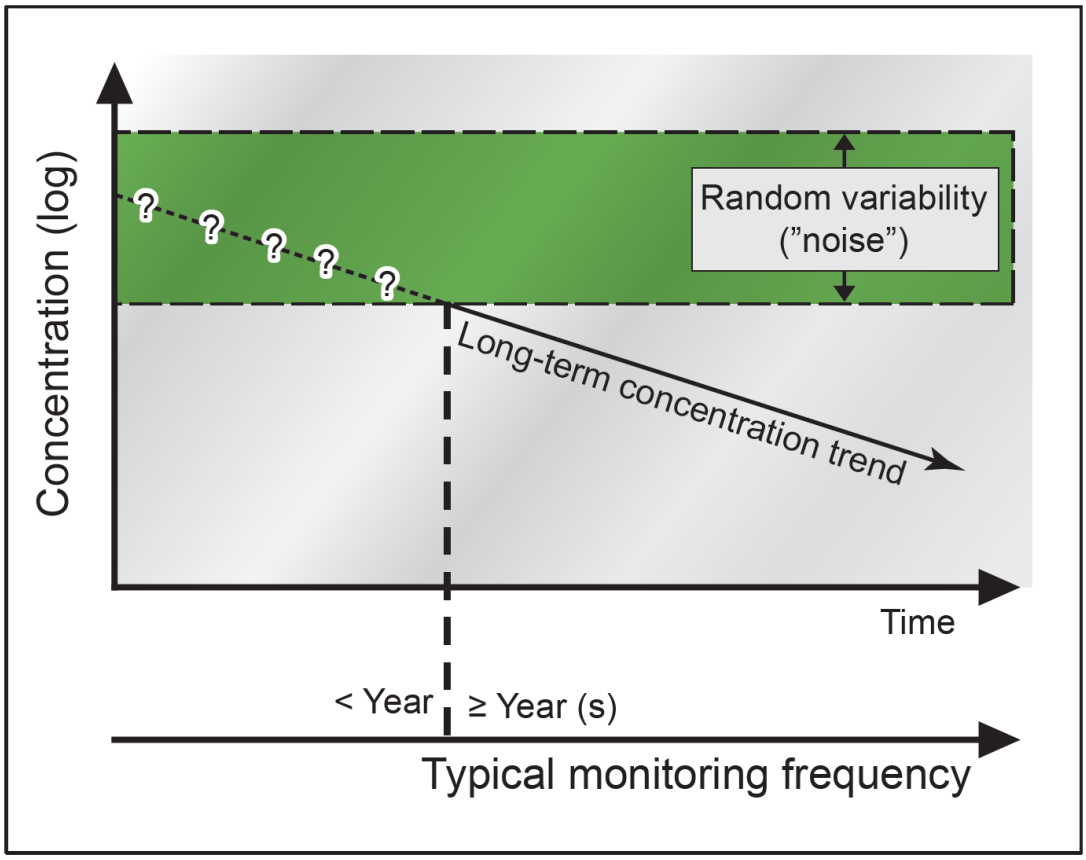


Figure A6.3: Conceptual illustration of monitoring frequency required to characterise long-term concentration trends (after McHugh *et al.*, 2015).

These findings are supported by other guidance regarding approaches to MNA performance (Wilson, 2011).

Several tools have been developed that can assist with determining the optimal performance monitoring frequency in critical plume zones (e.g. MAROS Software [Aziz *et al.*, 2000]; ITRC, 2013; McHugh, 2015). More advanced spatio-temporal approaches have recently emerged (e.g. McLean, 2018; Torres, 2019), that utilise geostatistical tools within a temporal framework to improve interpolation of groundwater concentration timeseries to identify temporal redundancy in monitoring data. GWSDAT (Jones *et al.*, 2014) implements the 2D method of McLean (2018), whereas deeper, complex systems may benefit from the 3D method proposed by Torres (2019).

Analytical Parameters

The analysis of samples collected during the performance monitoring programme should include laboratory and field parameters necessary to confirm that NA is occurring as predicted, and to ensure the MNA remedy is protective of receptors. The suite of parameters may be modified relative to those used during the initial demonstration of the appropriateness of MNA. As a minimum, the contaminants of concern, any degradation intermediates and end products should be routinely analysed, adequate to demonstrate contaminant mass loss or concentration reduction at plume scale.

Periodically, geochemical and molecular parameters (e.g. terminal electron accepting process-indicating parameters, degradation end products, stable isotope fractionation [CSIA], microbial abundance and functional gene expression [MBTs]) indicating secondary and tertiary lines of evidence for NA should be determined to confirm conditions continue to be conducive for principal attenuation processes to be effective, with no substantial reduction in attenuation rate.

Assessing Long-Term MNA Performance

MNA performance should be evaluated in regular review cycles (typically up to 5 years) to determine whether:

- Monitoring should continue according to the defined programme or if it needs to be revised, including change in the frequency of sampling, wells monitored and/or analytical suite;
- Intervention is required because MNA is not performing as expected; and
- Monitoring can cease.

The cost of the review process needs to be included in financial provisions for MNA.

To assist in the review and assessment of MNA performance, visualising monitoring data (Appendix 5) should support initial evaluations of spatial and temporal trends, compliance with remedial targets and potential adverse changes to expected plume behaviour. Statistical methods provide the most robust means to formally assess concentration changes and compliance with remediation targets in timeseries data. Statistical analysis could include (e.g. Wilson, 2011; Jones *et al*., 2014):

- Re-evaluating attenuation rates with consideration of uncertainty in the estimates (Figure A6.4);
- Assessing the significance of observed reductions in concentrations and the probability that reductions are adequate to meet remedial targets within a specified period.

These methods provide a statistically-based decision criterion, at some predetermined level of confidence, on which to:

- Cease monitoring
    - Contaminant concentrations in the plume have reached background levels; or
    - Remedial objectives have been met, and NA can be relied on to further reduce contaminant levels; or
    - Remedial objectives have been substantially met and falling trends in contaminant concentrations have been defined to the extent that there is a high degree of confidence that the remedial objectives will be achieved within an agreed MNA performance review period.
- Continue monitoring
    - Reductions in concentrations are statistically significant (i.e. MNA is performing as expected), but plume concentrations are unlikely to meet remedial objectives within the predetermined review period, therefore continue monitoring to establish if plume concentrations will achieve remedial objectives within the agreed timeframe for MNA.

- Trigger contingency planning
  - Concentrations are not changing or trends have reversed, MNA is ineffective and remedial targets will not be met within the agreed timeframe for MNA.
  - If rates of NA are significantly slower than expected and MNA is unlikely to achieve remedial objectives within the time agreed for the MNA programme.

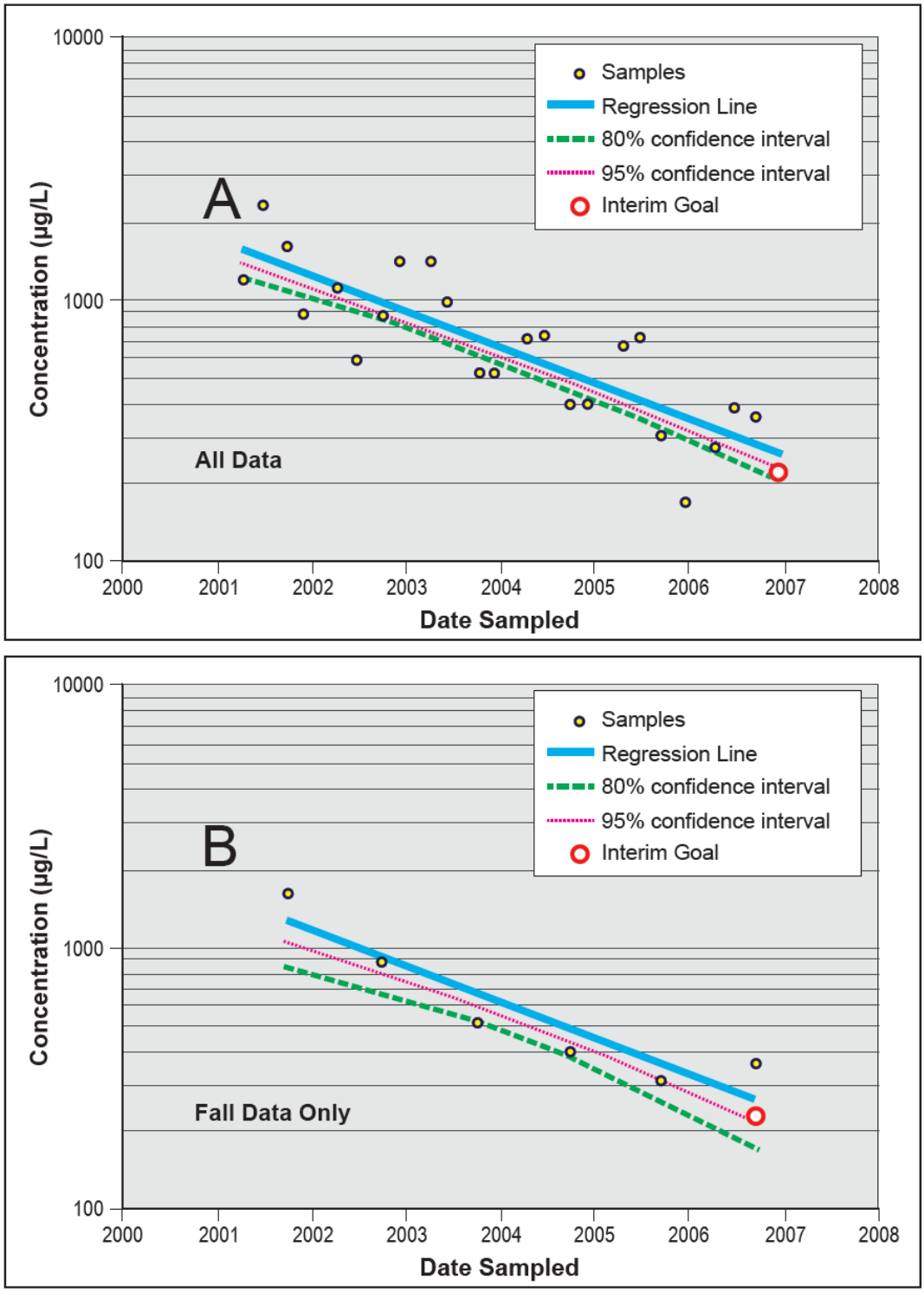


Figure A6.4: Re-evaluating attenuation rates with consideration to uncertainty in the estimates (Wilson, 2011).

Contingency Plan

The performance monitoring strategy should include a Contingency Plan, to govern additional measures to be implemented if MNA proves to be ineffective or insufficient as a risk management strategy.

The Contingency Plan should include:

- The decision criteria on which it will be triggered;
- Which stakeholders should be notified and involved in the decision-making process;
- Review of the conceptual model to understand what factors may have caused the reduction in MNA performance demonstrated in earlier steps of the process; and
- Outline measures that will be implemented and the timescale over which these measures may be implemented.

Criteria for triggering the Contingency Plan may include the following:

- Imminent risk of impact at the receptor;
- Concentration change relative to remedial target and/or trend reversal in monitoring wells or at plume scale exceeding a specified threshold value, for example, due to new releases of contaminants to groundwater and plume expansion;
- Contaminant concentrations are not decreasing at a sufficient rate to meet remedial objectives within the desired timeframe;
- Changes in groundwater or land use adversely influencing the effectiveness of NA and/or ability to monitor NA using the monitoring network; and
- Non-technical issues (e.g. operator goes into administration).

A6.3 Ceasing Monitoring

MNA performance monitoring can cease once remediation objectives have been met or concentration trends are understood with adequate confidence that verify objectives are expected to be met within an agreed period.

Monitoring data collected to demonstrate MNA may also be used for performance monitoring to indicate long-term trends. It is therefore plausible that concentration trends are characterised to an adequate level of confidence within the first review period following implementation of performance monitoring.

Regulatory approval should be sought to cease monitoring. The case for ceasing MNA will be provided by the conceptual model demonstrating the long-term effectiveness of NA at protecting receptors.

Once approval to cease monitoring has been provided, it is recommended that the performance monitoring network is decommissioned to mitigate risk of recontamination from the surface via deteriorating, unsealed wells.

Appendix 7: Groundwater Flow and Transport Models

A7.1 Introduction

Groundwater flow and transport models are useful tools to assist the demonstration of NA and evaluate future performance of MNA. A groundwater flow and transport model can be used in two fundamental ways:

- To understand how current conditions evolved;
- Predict future conditions and MNA performance under these conditions.

With regard to MNA, modelling approaches provide means to integrate and consider variability in complex and often diverse data collected during characterisation and performance monitoring stages, quantitatively assess and confirm the main attenuation processes, then forecast MNA performance with consideration of factors that may influence its effectiveness and assess whether remedial objectives are likely to be met.

Modelling can provide a means to confirm the conceptual model for NA (i.e. whether simulation of the conceptual model matches observation data) and provides a rigorous framework for identifying data gaps and uncertainties. A conceptual model will be required to develop an initial groundwater model, that then informs refinement and improvements to the conceptual model. The conceptual model and groundwater models should work iteratively to develop quantitative understanding of key hydrogeological and biogeochemical processes on which to base predictions and decision making (*Step 2 Field Demonstration*), including:

- Contaminant concentrations at receptors and arrival times;
- Source lifetime;
- Plume extent and dynamics (expanding, stable, shrinking);
- Plume concentration trends and rate of change;
- Quantification of transport and reactive transport parameters (e.g. degradation rates, sorption coefficients, dispersivity coefficients, and processes influencing contaminant attenuation);
- Monitoring locations and sampling frequencies; and
- Providing a tool for effective communication with stakeholders, in particular regulators.

In addition to confirming the effectiveness of NA assuming continuation of current conditions, scenarios that might be considered to assess the long-term performance of MNA as part of *Step 3 Predictive Modelling* could include:

- Reduction in the rate of attenuation due to adverse change in geochemistry, such as accumulation of cis-DCE or VC during reductive dechlorination stall or saturation of adsorption sites;
- Change in land use or receptor characteristics, resulting in modification of the flow regime (e.g. increase in abstraction rate at public water supply well or change in meteoric recharge rates);

- Potential for recontamination of the aquifer due to back diffusion from low permeability storage zones; and
- Effects of climate change, such as changing water table/flow regime.

Effective application of models to support MNA evaluation will require technical expertise and comprehensive understanding of governing flow and transport processes. Groundwater modelling can and must be performed transparently if the results are to be relied upon. Similarly, modelling applications that support MNA evaluation must be reported clearly and coherently to instil confidence in stakeholders (including the regulator), and include explanations of the assumptions and limitations of the modelling approach(es) applied, how these influence predicted outcomes and inform the Stage 2 and 3 conceptualisation of MNA. The basic steps in the selection, use and reporting of flow and transport models are provided in UK and international guidance (McMahon *et al*., 2001a; McMahon *et al.,* 2001b; McMahon *et al.,* 2001c; Reilly and Harbaugh, 2004; Barnett *et al.,* 2012)).

This appendix does not aim to provide modelling guidance. Rather it introduces the types of model currently available for modelling MNA and considerations for transport model applications.

A7.2 Model Selection

In designing and applying a solute transport model to MNA, the purpose and expectations of the model need to be defined (i.e. what questions should it answer and to what level of confidence). The selection of the model should be driven by the complexity of the site and potential source-pathway-receptor linkages. Inappropriate selection and/or use of models may give rise to erroneous conclusions, be time consuming and costly.

Two basic types of model are available – analytical and numerical models:

Analytical models are capable of solving the general transport equation with specific limitations.

Analytical models such as the Environment Agency's Remedial Targets Worksheet (Environment Agency, 2006), BIOSCREEN (Newell *et al.,* 1996), BIOCHLOR (Aziz *et al.,* 2000; Aziz and Newell, 2002), NAS (Mendez *et al.,* 2004), CoronaScreen (Wilson *et al.*, 2005), REMChlor (Falta, 2008) and REMChlor-MD (Farhat *et al.,* 2018) have been established specifically for use in modelling NA and/or MNA (refer to Table A7.1).

The solution technique typically requires assumptions of uniform hydraulic properties throughout the domain, uniform steady-state groundwater flow (in some cases limited to one-dimensional advection), simple boundary conditions, simple source geometry, first-order contaminant transformation with rates constant within a defined area (in some cases for a single decay pathway) and uniform linear equilibrium partitioning.

Analytical models can be useful in providing estimates of contaminant migration for plumes where these assumptions can be technically supported based on the site conditions, for instance a plume with a well-defined contaminant source within a relatively homogeneous, thin aquifer that is bounded by aquitards or an aquitard and the

water table where the aquifer has relatively constant geochemical conditions throughout the plume.

Analytical models provide valuable assessments of simple sites or screening-level assessments of more complex conditions. The advantages of these models are that they are often simpler to use and have fewer data requirements compared to numerical models. Multiple simulations can be run relatively quickly to evaluate, in broad terms, the range of potential outcomes. Despite their ease of use, the limitations of analytical models must be recognised, in particular for insufficiently characterised and/or complex hydrogeological situations which could generate misleading results. Additional analysis to explore the sensitivity of analytical models to site data is recommended to better understand how these variables influence uncertainty in predicted outcomes and ultimately decisions made concerning MNA.

Multi-dimensional reactive transport **numerical models**, often capable of simulating multiple contaminant species simultaneously, discretise the transport equation, which is solved iteratively within a defined numerical domain. Numerical models allow for more detailed configuration of the model domain to more closely match site features and, therefore, have advantages over analytical models for some sites.

Numerical models may be needed when site conditions cannot be described under the simplified flow, reaction, or adsorption process assumptions required for use of some analytical models, for example:

- The groundwater flow system at a site may not be uniform spatially and/or temporally because of a complex distribution of hydraulic conductivity, complex recharge/discharge elements, or transient flow conditions;
- Sources distributed in multiple locations, multiple contaminant species with multiple reaction pathways, and multiple oxidation/reduction conditions within the plume area cause complexities in modelling the reaction processes at a site;
- Linear equilibrium sorption is not appropriate in some cases depending on the nature of the contaminant and the aquifer solids; and
- NA processes are dependent on contaminant speciation, sensitive to transient oxidation/reduction conditions, include reactions such as precipitation and ion exchange and/or can be described by more complex kinetic models (e.g. Monod kinetics).

Numerical models are more appropriate for site conditions that include any, or all, of the above complexities. The modular three-dimensional code MODFLOW (McDonald and Harbaugh, 1988; Harbaugh *et al*., 2000; Harbaugh, 2005) is used determine groundwater flow (in 3D), and provides the platform for simulating transport and reactive transport under these more complex conditions. Examples of transport model codes are MT3D, MT3DMS and MT3D-USGS (Zheng, 1990; Zheng and Wang, 1999; Bedekar *et al*., 2016). Reactive transport codes include RT3D (Clement, 1997) and PHT3D (Prommer and Post, 2010). Descriptions of these numerical models are provided in Table A7.1.

Similar to analytical models, numerical models have limitations in how they can be configured to match site conditions. Equations cannot describe all of the nuances for each term within the transport equation. Numerical models cannot therefore reproduce

reality but can be configured to more closely match the site conditions and processes than analytical models.

Most analytical and numerical models are deterministic, that is, use a single value to define each model parameter, and the result is a single number. Although running deterministic models multiple times can indicate the effects of varying individual parameters (e.g. variation in hydraulic conductivity) on model predictions, stochastic approaches can provide more efficient and comprehensive insights on prediction uncertainty, including parameter covariance. Stochastic models that assess variable parameters using ranges or probability density functions (e.g. normal, lognormal etc.) within a Monte Carlo framework include ConSim (Golder, 2018), PREMChlor (Liang *et al*., 2010), and, for numerical models (MT3DMS, RT3D etc.), via PEST and some commercially-available graphical user interfaces (GUIs). More advanced stochastic approaches aim to reconcile spatial and/or temporal heterogeneity through geostatistical methods and estimate the uncertainty in predictions (range or distribution) based on these variables using probability theory (Renard, 2007). Despite recent advances to consider variability/heterogeneity more in site evaluations, application of these more advanced stochastic approaches, are not standard practice, but this will undoubtedly change with time (Konikow, 2011) and increasing accessibility to these methods (e.g. via the PEST and PEST++ suites).

A7.3 Calibration and Prediction

The reliability of a model may be improved with calibration of variable parameters (e.g. hydraulic conductivity or degradation rate) to match modelled with observed flow and concentration data. However, it should be recognised that accurate modelling of subsurface solute transport processes at plume scale is challenging. A numerically accurate solution is often expected but all models are a simplification of reality; conceptual weaknesses in the underlying theory, governing transport equation and mathematical solutions, plus limitations associated with sufficiency of reliable data on which to base the model, will inevitably introduce some errors (Konikow, 2011). Awareness of the sources of error will help model users minimise and account for this when interpreting model results. Experience indicates that some (if not most) of the difficulties with transport models arise from errors, inadequacies and uncertainties in the data used to estimate parameters. As such, transport models should be expected to reproduce major trends or locally average values rather than be expected to accurately match all variations observed in the field data (Konikow, 2011).

Groundwater flow is simpler to simulate than solute transport or reactive transport, although flow and transport are inherently linked. The more accurately and precisely the flow velocity field can be simulated, the less uncertain transport modelling should become. There are practical limits to how well heterogeneity and the flow velocity field can be defined. Good judgment and sensitivity analyses may help in balancing costs and benefits and in deciding when existing data are sufficiently good.

Solute transport models are often “highly parameterised”. Highly parameterised models are characterised by having more parameters than can be estimated uniquely for a given calibration dataset. Non-unique solutions can create calibration difficulties in that there may be a number of possible combinations of transport parameter values that match the calibration dataset. “Regularised inversion” is a mathematical approach that provides a

means of obtaining a unique calibration from the range of fundamentally non-unique, highly parameterised model calibrations (Doherty and Hunt, 2010; Doherty *et al.*, 2010a). "Regularisation" simply refers to approaches that make non-unique problems mathematically tractable; "inversion" refers to the automated parameter-estimation operations that use observation data to constrain model input parameters (Hunt *et al.*, 2007). Regularised inversion problems are commonly addressed by the use of the parameter estimation codes PEST and PEST++.

While non-uniqueness may be unavoidable in solute transport modelling, performing multiple calibrations of the model can assist in understanding how prediction uncertainty is influenced by estimated parameters matching the calibration data. One of the most significant trends in groundwater modelling over the past two decades has been the shift in focus from "model calibration" to "calibration-constrained model predictive uncertainty analysis". This shift in emphasis recognises the fact that groundwater models are built to make predictions that support the making of important management decisions, such as whether to implement MNA. These predictions are often accompanied by a large amount of uncertainty that should be quantified to allow evaluation of the risks associated with site management strategies (Doherty, 2015). Procedures for performing parameter and predictive uncertainty analysis are provided by USGS (Doherty *et al.*, 2010b) and implemented using the PEST++ suite.

Some reactive transport modelling approaches are capable of integrating data provided by advanced tools such as compound specific isotope analysis (CSIA, Appendix 8) and/or molecular biological tools (MBTs, Appendix 9) with more typical observation data (e.g. groundwater elevations, contaminant concentrations). The application of this class of models can provide greater confidence in the interpretation of these data and support the development of combined lines of evidence for NA (primary, secondary and tertiary).

Modified analytical models, such as BIOCHLOR-ISO (Höhener, 2016) and Bioscreen-AT-ISO (Höhener *et al.*, 2017), have been developed to simulate contaminant transport in simple hydrogeological systems combining chemical analytical data with isotopic fractionation data based on the simplifying assumptions of the Rayleigh model (Appendix 8). CSIA and MBT data can be difficult to interpret at sites with more complex hydrogeology, biogeochemistry or release histories. More advanced reactive transport models (e.g. PHREEQC, PHT3D or RT3D) provide the capability to meaningfully integrate these data through definition of degradation pathways using stable isotope geochemistry and/or advanced kinetic models describing microbial activity and population growth.

The research undertaken for prediction of chlorinated solvent bioremediation for the UK SABRE project provides an indication of the extent to which modelling can extend understanding of complex problems, incorporating diverse datasets often collected during MNA (CL:AIRE, 2010b). Table A7.1 identifies analytical and numerical models with application to MNA studies. This table is not intended to be comprehensive and other models may be more appropriate in particular situations.

Table A7.1: Analytical and numerical models with application to MNA studies.

Model/Code	Type	Description	Reference or Source
BIOCHLOR	Analytical-deterministic	BIOCHLOR is a screening model that simulates NA of dissolved chlorinated solvents. Based on the Domenico analytical solute transport model, simulates 1D advection, 3D dispersion, linear adsorption and sequential degradation assuming first-order decay. It assumes a homogeneous isotropic aquifer with uniform regional flow.	United States Environmental Protection Agency (USEPA) https://www.epa.gov/water-research/biochlor-natural-attenuation-decision-support-system
BIOSCREEN	Analytical-deterministic	BIOSCREEN is a screening model that simulates NA of dissolved hydrocarbons. The model is designed to simulate biodegradation by both aerobic and anaerobic reactions. BIOSCREEN is based on the Domenico analytical solute transport model and allows for advection, dispersion, adsorption, both aerobic decay and anaerobic reactions. Biodegradation can be modelled as a first-order decay process or instantaneous reaction with electron acceptors (dissolved oxygen, nitrate and/or sulfate).	USEPA https://www.epa.gov/water-research/bioscreen-natural-attenuation-decision-support-system
BIOSCREEN-AT	Analytical-deterministic	BIOSCREEN-AT is an enhancement of the standard BIOSCREEN program that can implement an exact three-dimensional analytical solution for solute transport from a patch boundary condition within a semi-infinite aquifer. BIOSCREEN-AT simulates advection, dispersion, adsorption, both aerobic decay and anaerobic reactions. Biodegradation can be modelled as a first-order decay process or instantaneous reaction with electron acceptors (dissolved oxygen, nitrate and/or sulfate).	SS Papadopoulos https://www.sspa.com/software/bioscreen

Model/Code	Type	Description	Reference or Source
ConSim 2.5	Analytical-stochastic	ConSim is a screening level tool that can be used within the quantitative risk assessment framework provided by the Environment Agency's Remedial Targets Methodology (Environment Agency, 2006). ConSim is used to assess the potential for leaching of contaminants from multiple sources, migration towards one or more receptors and attenuation in the unsaturated zone and an aquifer. Dilution, sorption and biodegradation/decay may be incorporated. Prediction uncertainty is taken into account through the use of parameter input ranges and a Monte Carlo probabilistic calculation methodology.	Golder http://www.consim.co.uk/
CoronaScreen	Analytical-deterministic	CoronaScreen is a package of three spreadsheet-based analytical screening models for the performance assessment of NA in groundwater. The models have a different conceptual framework and mathematical formulation for specific contaminant scenarios. The models simulate the evolution of contaminant plumes in groundwater in terms of the spatial distribution of (plume "fringe" and plume "core") biodegradation processes that occur over time. The models offer the possibility to estimate the maximum plume length, the time to achieve this, the plume biodegradation rate and contaminant concentration at a given compliance point. The models can be used to evaluate mixed plumes of organic and inorganic chemicals, using standard site characterisation information and groundwater quality data collected from a relatively simple network of single screen monitoring wells and multilevel sampling wells.	Groundwater Protection and Restoration Group, University of Sheffield https://www.sheffield.ac.uk/gprg/technology/coronascreen

Model/Code	Type	Description	Reference or Source
MT3D MT3DMS MT3D-USGS	Numerical-deterministic (stochastic capability with some GUIs)	The MT3D family of transport codes were first released in 1990 as MT3D for single-species mass transport. Two updated versions have since been released: MT3DMS and MT3D-USGS. MT3DMS is a numerical multispecies contaminant transport model with a modular structure simulating solute transport processes (advection, dispersion, linear and non-linear sorption, first-order decay/degradation) independently or jointly. MT3DMS interfaces directly with the United States Geological Survey (USGS) finite-difference groundwater flow model, MODFLOW. MT3DMS contains several transport solution techniques, including the fully implicit finite-difference method (FDM), the particle-tracking based method of characteristics (MOC) and its variants, and a third-order total-variation-diminishing (TVD) scheme that conserves mass while limiting numerical dispersion and artificial oscillation. MT3D-USGS is a USGS updated version of MT3DMS, that includes additional transport modelling capabilities such as unsaturated zone processes, surface water interactions, inter-species and sequential reactions, separate specification of sorption coefficients in mobile and immobile zones.	University of Alabama http://hydro.geo.ua.edu/mt3d/ USGS https://www.usgs.gov/software/mt3d-usgs-groundwater-solute-transport-simulator-modflow
NAS	Analytical-deterministic	Natural Attenuation Software (NAS) is a screening tool to estimate remediation timeframes for MNA to lower groundwater contaminant concentrations to regulatory limits, and to assist in decision making on the level of source zone treatment in conjunction with MNA using site-specific remediation objectives.	USGS Virginia Polytechnic Institute and State University (Virginia Tech) Naval Facilities Engineering Command (NAVFAC) https://www.nas.cee.vt.edu/index.php

Model/Code	Type	Description	Reference or Source
NAS (cont.)		NAS consists of a combination of analytical and numerical solute transport models. Natural attenuation processes that NAS models include advection, dispersion, sorption, NAPL dissolution, and biodegradation. NAS determines redox zonation, and estimates and applies varied biodegradation rates from one redox zone to the next.	
PHT3D	Numerical-deterministic (stochastic capability with some GUIs)	PHT3D integrates geochemical and transport modelling by coupling MT3DMS (Zheng and Wang, 1999) with PHREEQC-2 (Parkhurst and Appelo, 1999) for simulation of complex multicomponent reactive transport processes. PHT3D is capable of simulating equilibrium-controlled aqueous complexation / speciation, kinetic reactions of aqueous components such as biodegradation of organic compounds, mineral precipitation / dissolution, ion exchange, and surface complexation reactions.	University of Western Australia, Flinders University School of the Environment (South Australia) and National Centre for Groundwater Research and Training http://www.pht3d.org
PREMChlor	Analytical-stochastic	The Probabilistic Remediation Evaluation Model for Chlorinated solvents (PREMChlor) simultaneously evaluates the NA of source and plume considering the uncertainties in all major parameters, thereby supporting the demonstration of MNA, or selection of remediation alternatives. PREMChlor simulates plume NA spatially (2D) and temporally for parent and daughter compounds, based on advection with dispersion, sorption and sequential first-order decay. Probabilistic functionality is provided by coupling REMChlor with GoldSim, a Monte Carlo modelling package, and representing variable parameters with probability density functions.	Strategic Environmental Research and Development Program (SERDP) Environmental Security Technology Certification Program (ESTCP) https://serdp-estcp.org/projects/details/5bd87a57-f0f7-4f11-a2ab-dd5213b5bf4a/er-200704-project-overview

Model/Code	Type	Description	Reference or Source
REMChlor	Analytical-deterministic	REMChlor, or Remediation Evaluation Model for Chlorinated solvents, is an analytical solution for simulating the transient effects of groundwater source and plume remediation. The contaminant source model is based on a power-function relationship between source mass and source discharge, implicitly simulating matrix diffusion in the source. It can consider partial source remediation at any time after the initial release. The source model serves as a time-dependent, mass-flux boundary condition to the analytical plume model, where flow is assumed to be one dimensional. The plume model simulates first-order sequential decay and production of several species. The decay rates and parent/daughter yield coefficients are variable functions of time and distance.	USEPA https://www.epa.gov/water-research/remediation-evaluation-model-chlorinated-solvents-remchlor
REMChlor-MD	Analytical-deterministic	REMChlor-MD is a new version of the REMChlor model with the ability to simulate matrix diffusion processes in the source and plume. REMChlor-MD is a Microsoft Excel-based management tool for addressing contamination in a broad range of geological settings, including fractured porous media, heterogeneous media with low permeability inclusions, and high permeability zones that are adjacent to low permeability aquitards. The toolkit allows the accounting of several types of source and plume remediation activities.	GSI Environmental Environmental Security Technology Certification Program (ESTCP) https://www.gsi-net.com/en/software/free-software/remchlormd.html
Remedial Targets Methodology Worksheet v3.2	Analytical-deterministic	The Remedial Targets Methodology worksheet is an Excel-based screening level model that implements the Environment Agency's Remedial Targets Methodology for hydrogeological risk assessment of land contamination (Environment Agency, 2006).	Environment Agency https://www.gov.uk/government/publications/remedial-targets-worksheet-v22a-user-manual

Model/Code	Type	Description	Reference or Source
Remedial Targets Methodology Worksheet v3.2 (cont.)		This model simulates leaching, dilution, dispersion, sorption and first-order decay of a polar or non-polar solute from a single source within a single aquifer unit. Steady-state and transient solutions are simulated to assess concentrations in groundwater at a downgradient point of compliance and back-calculate remedial target concentrations in soil or groundwater based on a specified water quality standard.	
RT3D	Numerical-deterministic (stochastic capability with some GUIs)	RT3D is a 3D multispecies reactive transport model for solutes in groundwater. RT3D couples MT3DMS (Zheng and Wang, 1999) with several pre-programmed reaction modules for common biologically mediated reactions, rate-limited sorption, NAPL dissolution, kinetic-limited degradation using multiple electron acceptors, Monod kinetics and others. Furthermore, RT3D permits users to add any reaction kinetics desired/suitable to represent multiple chemical species in aqueous and sorbed phases.	Pacific Northwest National Laboratory https://www.pnnl.gov/

Appendix 8: Compound Specific Isotope Analysis (CSIA)

A8.1 Introduction

The selection of MNA as a viable remedy for a site may require an evaluation of the contribution of natural biodegradation or abiotic transformation processes within groundwater. However, demonstrating unequivocally that a contaminant is being degraded in the environment is challenging. Concentration-based methods demonstrating primary and secondary lines of evidence for MNA, such as the presence of intermediates and favourable geochemical conditions, may be confounding, particularly for those contaminants which degrade slowly and/or whose degradation pathways produce non-unique or non-persistent by-products or end products (USEPA, 2008). Furthermore, they provide little information about the processes responsible for removal of a specific contaminant, and cannot distinguish degradation from other physical processes (e.g. dilution/dispersion), which can reduce concentrations but not the contaminant mass.

Compound Specific Isotope Analysis (CSIA) is an environmental molecular diagnostic technique that can assess the ratio of heavy to light stable isotopes of selected elements within contaminants as well as metals in environmental samples (USEPA, 2008). For example, ^{12}C is the most common carbon isotope in naturally-occurring organic compounds but a small fraction of the heavier ^{13}C will also be present. Instrumentation capable of performing CSIA measurements was introduced in 1997 for chlorinated ethenes. Between the year 2000 and 2010, the technique has steadily become applicable to further chlorinated and non-chlorinated hydrocarbons, as well as nitrobenzenes/anilines and more recently (~2010), pesticides and metabolites, and polar organic micropollutants. Figure A8.1 shows the timeline of CSIA development, its maturation and the range of contaminants to which the technique can now be applied.

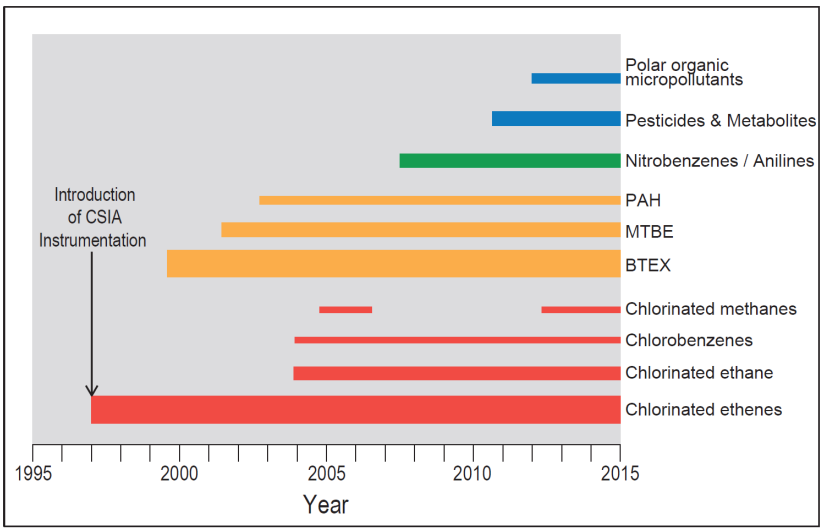


Figure A8.1: The development of CSIA and range of contaminants to which the technique can be applied (Source: Geosyntec).

A number of reviews have focused on principles and applications of CSIA (Zimmermann *et al*., 2020; Cui *et al*., 2021; Alberti *et al*., 2017). The elements that make up chemical compounds have distinct isotopic ratios, which are a function of the manufacturing process and degradation of the compound after it has been released to the environment. The ratios in manufactured chemicals are generally well known to within a few percent. When organic contaminants degrade in the environment, the ratio of stable isotopes begins to change. CSIA can precisely measure the small changes in isotopic ratios, providing unequivocal evidence that degradation has occurred and furthermore can potentially identify the degradation process and estimate the extent and rate of degradation (USEPA, 2008).

A8.2 Applications

From a NA standpoint, the shift in isotopic ratios measured by CSIA can be useful in providing the following (USEPA, 2008):

1. Demonstrating that a parent compound is being degraded;
2. Differentiating between destructive and non-destructive attenuation processes;
3. Differentiating between destructive pathways (e.g. anaerobic vs aerobic vs abiotic);
4. Estimating the extent of degradation;
5. Estimating the rate of degradation; and
6. Source identification and differentiation.

The technique can also be applied to environmental forensics and assessing abiotic degradation as a result of chemical oxidation techniques, however, these are outside the scope of this document. Abiotic degradation of chlorinated solvents on reactive iron mineral surfaces also naturally cause measurable fractionation (Liang *et al*., 2007).

A8.3 Scientific Basis

Isotopes of elements have the same number of protons and electrons, but a different number of neutrons. For example, carbon has two stable isotopes; ^{12}C which contains six protons and six neutrons, and ^{13}C, which contains six protons and seven neutrons. As a result of the additional neutron in ^{13}C, it is heavier, and forms very slightly stronger chemical bonds. The quotient of heavy to light isotopes is called the isotopic ratio. The ratios present within chemicals naturally vary according to the source of feedstocks used in the manufacturing process, and the ratios present in the resulting formulations may differ from one batch to another.

During the process of biodegradation or abiotic degradation, the lighter isotopes degrade preferentially over those which are heavier. This is due to slight differences in the reaction rates of molecules with light isotopes compared to the heavy isotopes (a phenomenon known as kinetic isotope effect [USEPA, 2008]). Enrichment of the heavier isotope in the undegraded compound causes a shift in isotopic ratio relative to the isotopic ratio of the compound source, which becomes more pronounced as biodegradation or abiotic degradation proceeds (ITRC, 2011). This enrichment is referred to as isotopic fractionation. Organic metabolites produced during biodegradation are isotopically lighter than the parent compounds, due to the isotope fractionation. This enables organic biodegradation products to be linked to specific parent compounds and

pathways and is important when interpreting the biodegradation of organic compounds in mixtures.

An illustrated example how isotopic enrichment occurs along a groundwater flow path in which biodegradation of TCE is occurring is shown in Figure A8.2.

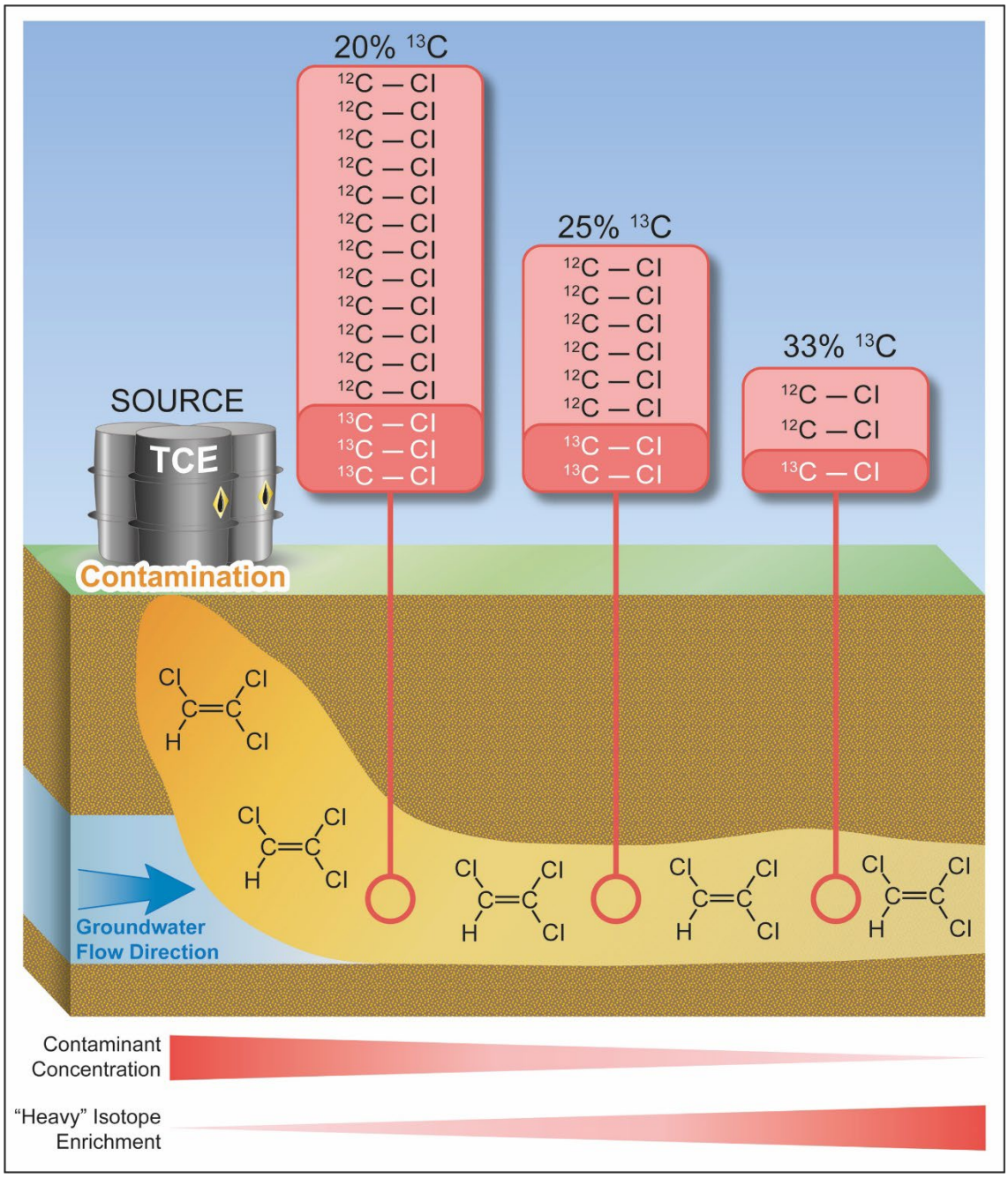


Figure A8.2: Illustration of ^{13}C enrichment during reductive dechlorination of TCE with a C-Cl bond. Adapted from ITRC (2011).

Characterisation of the isotopic ratios present within a well-defined source zone will improve confidence that the relative enrichment of isotopes downgradient are the result of degradation. If there is more than one source of contamination, it is possible that the isotopic ratios may differ between plumes, and give a false impression of biodegradation. Quantification of the isotope ranges from each of the sources is required.

In cases where biodegradation or physical destruction of the TCE molecules were not occurring, no isotopic enrichment would be apparent throughout the plume, despite reducing concentrations in groundwater with distance from the source due to physical attenuation processes.

In most instances transport and partitioning of contaminants in groundwater will not mask the relatively large isotopic fractionation due to biotic degradation (ITRC, 2011). Isotopic fractionation that occurs during volatilisation, dissolution, diffusion and sorption has been found to be relatively small and indiscernible in natural systems outside of the typical analytical uncertainty (USEPA, 2008; Adamson and Newell, 2014).

CSIA measures ratios of one or more stable isotopes in molecules and compounds. The technique is most commonly applied to carbon isotopes ($^{13}C/^{12}C$) in organic contaminants and is generally applied to compounds that contain ten or fewer carbon atoms, such as BTEX, MTBE, naphthalene, some chlorinated ethenes and ethanes. However, it can also be applied to hydrogen ($^{2}H/^{1}H$), nitrogen ($^{15}N/^{14}N$), oxygen ($^{18}O/^{16}O$ and $^{17}O/^{16}O$), chlorine ($^{37}Cl/^{35}Cl$), sulfur ($^{34}S/^{32}S$) and others. The measured isotopic ratios are normalised with respect to international isotopic standard reference materials and expressed in delta notation (e.g. $\delta^{13}C$) (USEPA, 2008).

CSIA can also be used to interpret the biodegradation of contaminants at low concentrations. Bennett (2017) reported that 1,4-dioxane present at 1 µg/l within groundwater was efficiently sorbed through the addition of a synthetic hydrophobic carbonaceous material to the sample. The 1,4-dioxane was recovered from the dried sorbent by thermal desorption within a gas chromatograph, and then analysed for isotopic ratios of both carbon and hydrogen. It is possible that this method could be applied to other contaminants that occur at low concentrations within the environment to extend the applicability of CSIA.

A8.4 Quantitative Interpretation of Isotope Data

For many organic contaminants, the relationship between isotopic fractionation and the extent of degradation is described by the Rayleigh model. The Rayleigh model states that isotopic fractionation is proportional to the change in concentration, with the proportionality constant expressed as the isotopic enrichment factor (ε). If the degradation pathway is known, literature or laboratory/microcosm-derived ε values can be used to estimate the fraction of contaminant remaining after degradation (f), and the extent of degradation (USEPA, 2008).

For the carbon isotope system ($^{13}C/^{12}C$):

$$R_t = R_0 \, f^{(\alpha-1)} \qquad \text{Equation A8.1}$$

where R_t is the stable isotope ratio ($^{13}C/^{12}C$) of the compound at time t, R_0 is the initial isotopic ratio of the compound and f is the fraction of contaminant remaining, where f = 1 at t = 0 and decreases to f = 0 when the reactant compound is fully transformed to products. The stable isotope fractionation factor (α) is defined as:

$$\alpha = \frac{(1000 + \delta^{13}C_a)}{(1000 + \delta^{13}C_b)}$$ Equation A8.2

where subscripts *a* and *b* may represent a compound at time zero (t_0) and at a later point (t) in a reaction.

For degradation in groundwater:

$$f = e^{(\delta 13C groundwater - \delta 13C source)/\varepsilon}$$ Equation A8.3

where $\delta^{13}C_{groundwater}$ is the measure of the isotopic ratio in the organic contaminant in the sample of groundwater, $\delta^{13}C_{source}$ is the isotopic ratio in the non-degraded organic contaminant and epsilon (ε) is the stable isotope enrichment factor, defined as:

$$\varepsilon = (\alpha - 1).1000$$ Equation A8.4

The Rayleigh equation (Equation A8.5) may be used to predict the extent of biodegradation based on the changes in isotope ratios. It should be noted that application of the Rayleigh equation for intermediates in the degradation process (such as those resulting from reductive dechlorination of chlorinate ethenes) is not strictly possible.

$$\frac{(\delta_t + 1000)}{(\delta_o + 1000)} = \left(\frac{C_{Bt}}{C_0}\right)^{\frac{\varepsilon}{1000}}$$ Equation A8.5

Where*:*

δ_t = isotopic signature of the substrate at a time point

δ_0 = original isotopic signature of the substrate

C_{Bt}/C_0 = fraction of substrate remaining at time point t

ε = isotopic enrichment factor in ‰

The extent of biodegradation is often expressed as a percentage (B%) of the initial concentration using Equation A8.6:

$$B[\%] = \left(1 - \frac{C_{Bt}}{C_0}\right).100$$ Equation A8.6

Use of the above equations enables the quantification of contaminant biodegradation along a specified flow path or time interval. The amount of a contaminant degraded between the source, or starting point of observation, and a downgradient location (x) is described by Equation A8.7 (USEPA, 2008).

$$B[\%] = \left(1 - \frac{C_{Bx}}{C_0}\right).100 = \left[1 - \left(\frac{\delta_x + 1000}{\delta_0 + 1000}\right)^{\left(\frac{1000}{\varepsilon}\right)}\right].100$$ Equation A8.7

Based on the changes in isotopic ratios along a groundwater flow path identified by the Rayleigh equation (Equation A8.5), an *in situ* zero-order and first-order rate constant can be estimated.

In addition to demonstrating the extent of biodegradation of certain organic contaminants in groundwater, it is also possible to use the data to predict the extent and rate of degradation (USEPA, 2008). The rate of contaminant degradation can often be calculated from field data by evaluating observed decreases in contaminant concentrations with travel time along the aquifer flow paths. CSIA can be used to increase confidence in degradation rate estimates, by providing evidence that the reduction in concentration is due to destruction of the contaminant, and in many cases can provide a more reliable estimate of degradation rate (USEPA, 2008). A first-order rate coefficient is estimated using isotopic ratio data (e.g. $\delta^{13}C$) for a near source and downgradient location (or early and later time) with the isotopic enrichment factor (ε) from the Rayleigh model. Some analytical transport models are capable of simulating isotopic ratios in plumes that can assist in determining degradation rates by integrating CSIA with hydrogeological data and solute transport parameters (e.g. Höhener, 2016; Höhener *et al*., 2017).

The amount of degradation that needs to have occurred before CSIA, using $^{13}C/^{12}C$ isotopic ratios, can be confident in positively identifying biodegradation and estimating rates varies between compounds. For example, biodegradation of TCE can be detected at <20% degradation (USEPA, 2008). In contrast, aromatic hydrocarbons require 60% degradation to have occurred prior to positively identifying biodegradation, due to the more subtle carbon isotope fractionation that occurs. For petroleum hydrocarbons, analysis of both carbon isotope enrichment and hydrogen isotope enrichment can increase the sensitivity of the analysis to detect biodegradation as fractionation of $\delta^{2}H$ is greater than $\delta^{13}C$ for some petroleum hydrocarbons (Fischer *et al*., 2008; Gray *et al*., 2002).

CSIA can be difficult to interpret, especially at sites with complex hydrogeology or release histories (Kuder *et al*., 2014). Factors that can confound CSIA data and challenge application of the Rayleigh model at some sites include:

1. Mixed degradation processes (biotic and abiotic);
2. Contributions from multiple sources;
3. Formation and degradation of sequential transformation daughter products (e.g. cis-DCE and VC); and
4. Sorption and physical transport processes.

Dual or triple CSIA provides a means to deal with some of these factors. The application of these data in reactive transport models (e.g. PHREEQC, PHT3D or RT3D – Appendix 7) provides a way to strengthen the interpretation of CSIA alongside chemical analytical data and other hydrogeological data to develop clearer evidence for degradation processes.

A8.5 Sampling Technique

Sample collection for CSIA depends on the compounds of interest. For example, use of CSIA for the analysis of perchlorate in groundwater, sampling requires the pumping of

several hundred litres of groundwater through ion exchange columns to trap perchlorate (USEPA, 2008). For the analysis of carbon and hydrogen isotopes from volatile organic compounds, standard low-flow groundwater monitoring techniques can be used, with collection of samples into standard vials for volatile analysis. Some laboratories forgo the use of chemicals and rely on chilling of the samples post-collection for preservation, however, some recommend the addition of a preservative, typically 36% hydrochloric acid diluted 1:1 in water, resulting in a sample pH of <2. Other preservation methods are also used and the analytical laboratory should be contacted to ensure observation of suitable protocols for collecting, handling, and transporting the samples. A comprehensive guide to sampling is provided in USEPA (2008).

A8.6 Supporting Lines of Evidence and Summary

CSIA is considered a tertiary line of evidence in the assessment of NA (see Section A5.4). The greatest value from the information can be derived when it is used in conjunction with hydrogeological, geochemical and microbiological parameters. It is important to note that use of techniques to study biodegradation in groundwater that use an artificially labelled isotope (usually ^{13}C) will invalidate the use of CSIA. Small quantities of the artificially labelled isotope dissolve within the groundwater and alter the natural abundance of isotopes present within the contaminants. Due to the sensitivity of CSIA, the addition can skew the ratio of naturally-occurring isotopes sufficiently to result in inaccurate conclusions (USEPA, 2008).

Advantages of CSIA

- The technique is not dependent on trends in concentrations or daughter product generation;
- Allows very precise assessment of degradation of specific contaminants across a site;
- Multiple isotopes in a given molecule can be assessed, for example both $^{13}C/^{12}C$, $^{2}H/^{1}H$ and $^{37}Cl/^{35}Cl$ in TCE;
- Possible identification of source provenance; and
- Allows accurate *in situ* quantification of the extent of degradation and estimation of contaminant degradation rates.

Limitations of CSIA

- The applicability of the technique is limited for high molecular weight compounds such as petroleum hydrocarbons. This is because fractionation of an individual atom at the location of bond breakage due to biodegradation, may be masked by the presence of multiple copies of that atom at other locations within the molecule.
- For some compounds and degradation pathways, such as aerobic biodegradation of toluene, fractionation only occurs by reactions that break down the methyl group, rather than reactions that attack the benzene ring, which may result in an apparent limited amount of biodegradation. A combination of hydrogen and carbon isotope fractionation analysis should be performed.

Appendix 9: Molecular Biological Tools

A9.1 Introduction

Molecular biological tools (MBTs) are advanced and evolving techniques that analyse biological characteristics in soil and groundwater. MBTs provide strong but not definitive evidence to help understand, quantify and demonstrate the effectiveness of MNA. MBTs use deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) techniques to identify organisms potentially responsible for biodegradation and biological processes, their abundance and function. The theoretical basis and application of MBTs for MNA has been critically reviewed (Alvarez and Illman, 2005; Illman and Alvarez, 2009; Thornton *et al.*, 2016).

MBTs are technologies that can be applied to samples of environmental media and have supplanted microcosms in some cases. They provide an efficient and cost-effective means to collect spatially and temporally representative data supporting evaluations of biodegradation and other biologically-mediated processes. MBTs can therefore complement existing analyses for primary and secondary lines of evidence based upon comparable chemical, geochemical and biological datasets.

Over the past decade, some MBTs have achieved technological maturity and entered the commercial market for a broad range of groundwater pollutants. The rise in significance of MBTs in the MNA toolbox is well demonstrated by industry research that correlated five key parameters capable of demonstrating MNA for chlorinated ethenes[3], including the abundance of *Dehalococcoides sp.* (*Dhc*) (Lebrón *et al*., 2015), a group of microorganisms capable of complete dechlorination of PCE and TCE. More recent research has further highlighted the role of MBTs in the assessment of biodegradation potential, providing advanced understanding that could not otherwise have been gathered from conventional analyses alone (e.g. Badin *et al*., 2016; Ottosen *et al*., 2020; Ottosen *et al*., 2021; Toth *et al*., 2021). The quality of information afforded by these technologies has proven invaluable for demonstrating the feasibility of MNA in recent years and is readily accessible to industry via multiple commercial environmental DNA sequencing laboratories.

A9.2 Molecular Tools for MNA

An overview of microbiological methods used to interpret microbial communities and biodegradation processes in environmental samples is provided in Figure A9.1.

[3] Lebrón *et al*. (2015) demonstrated that the following parameters were correlated with the degradation rates of TCE, cis-DCE and VC: *Dehalococcoides sp.* [Dhc] abundance; magnetic susceptibility as a surrogate for magnetite abundance; iron sulfide (FeS); methane (CH_4); and ferrous iron (Fe(II)).

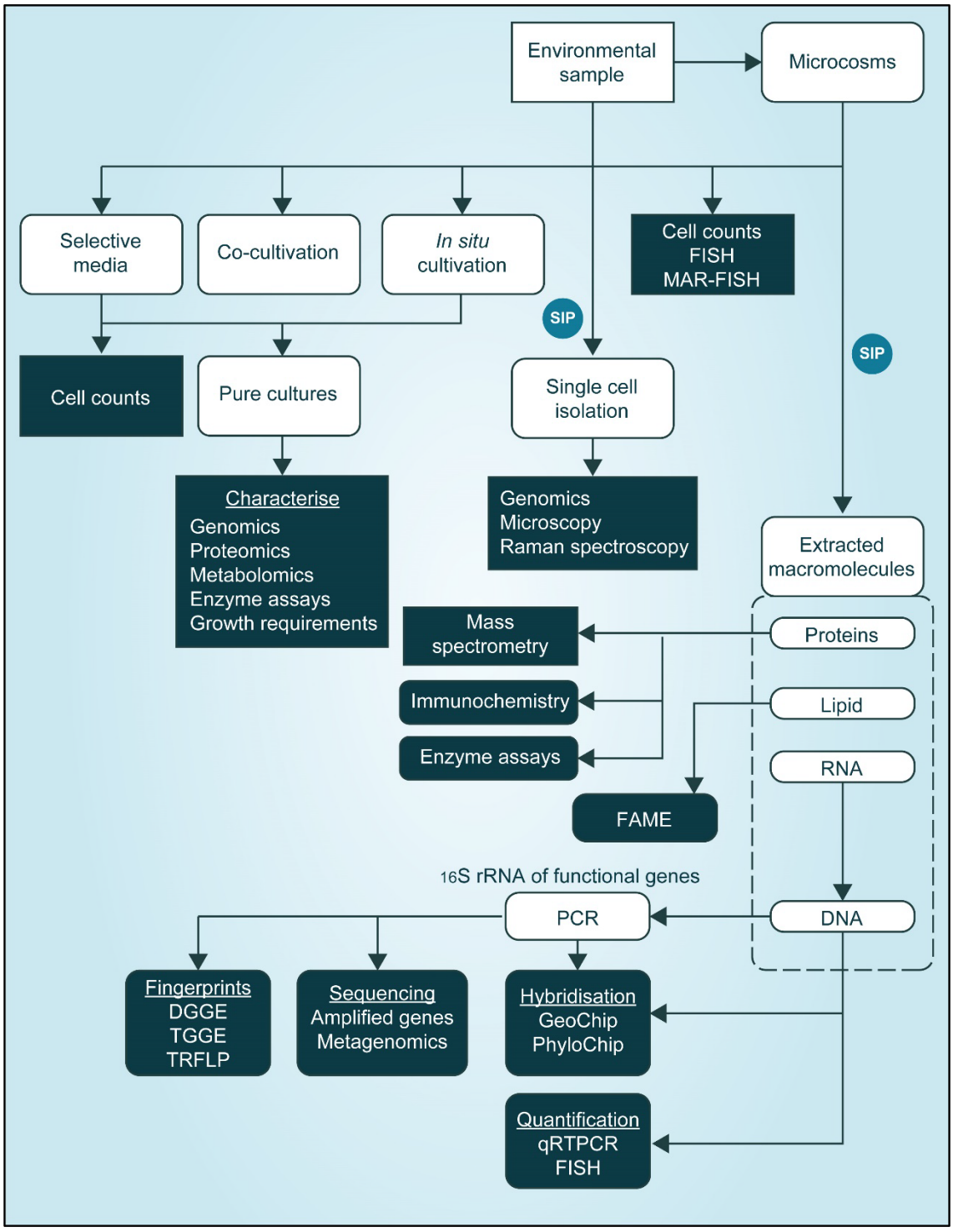


Figure A9.1: Overview of methods used to characterise microbial communities in environmental samples. SIP refers to stable isotope profiling. Reproduced from Thornton *et al*. (2016), Springer Nature, with permission of SNCNC.

MBTs using DNA/RNA-based methods are commercially available and are in common use to support contaminant and process-specific demonstration of biodegradation for MNA (e.g. ITRC, 2011; Adamson and Newell, 2014; NAVFAC, 2021):

- Show that key organisms responsible for biodegradation are present (e.g. *Dehalococcoides* for chlorinated ethene reductive dechlorination);
- Show that key enzymes indicating a specific biodegradation process are present and potentially active (e.g. VC reductase);
- Establish the relative abundance of key microbial populations.

These methods are described further in this appendix.

A9.2.1 Polymerase Chain Reaction and Variants

Polymerase chain reaction (PCR) is a technique that can test for the presence of a specific organism, family of microorganisms or expressed genes in environmental samples such as soil or groundwater. This technique can be used to identify microorganisms capable of degrading contaminants but not provide direct evidence alone that biodegradation has occurred or is occurring.

PCR utilises DNA from an environmental sample with DNA polymerase, DNA primers specific to a target 16S ribosomal RNA (16SrRNA) gene and DNA building blocks to synthesise and selectively amplify sequences of the 16SrRNA genes of interest in new strands of DNA.

PCR methods have been developed for a wide range of groundwater pollutants such as petroleum hydrocarbons, fuel oxygenates, phenols, pentachlorophenol, perchlorate, polychlorinated biphenyls (PCBs), metals, radionuclides and chlorinated solvents. PCR data for a specific gene or microorganism are usually reported simply as “present” or “absent”. However, these data can be used with variants of PCR and other MBTs to provide further insight on the abundance and activity of identified microorganisms.

Quantitative PCR (qPCR) measures fluorescence of specific dyes or “probes” that adhere to the PCR amplified DNA or genes, quantifying the number of specific sequences or genes from which the abundance of target microorganisms can be inferred.

Reverse transcriptase qPCR (RT-qPCR) utilises the production of RNA during biodegradation (tracked by specific enzyme production) to track the activity of target microorganisms. 16SrRNA is extracted from environmental samples then converted to “complementary” DNA (cDNA) that can be analysed by PCR to determine enzyme presence or measure enzyme abundance by qPCR.

qPCR and RT-qPCR data are usually reported in units of gene copies per litre. Collectively, there are currently approximately 50 qPCR and RT-qPCR target analyses in wide commercial use for MNA applications (much fewer than PCR) for chlorinated solvents and associated compounds (chlorinated ethenes [e.g. Clark *et al*., 2018], chlorinated ethanes [e.g. Scheutz *et al*., 2011], chlorinated methanes [e.g. Puigserver *et al*., 2020], chlorobenzenes [e.g. Qiao *et al*., 2018], chlorophenols [e.g. Mikkonen *et al*., 2018], chloropropanes [e.g. Yan *et al*., 2009], 1,4-dioxane [e.g. He *et al*., 2017a]), petroleum hydrocarbons (BTEX [e.g. Beller *et al*., 2008], PAHs [e.g. Oka *et al*., 2011]), fuel oxygenates such as MTBE [e.g. Kuder and Philp, 2008], PCBs [e.g. Liang *et al*., 2014] and select metals (e.g. uranium, [Barlett *et al*., 2012]).

Weatherill *et al*. (2018) cites several studies that show how qPCR methods enhanced understanding chlorinated solvent biodegradation pathways far beyond what could be determined from chemical and geochemical groundwater analysis alone. This includes the combined use of chemical analysis and qPCR to demonstrate co-occurrence and co-activity of aerobic VC degraders and anaerobic *Dhc* in riverbed sediments, where sharp redox gradients are often characterised (Atashgahi *et al*., 2017). VC degradation studies in aerobic and anaerobic microcosms provided geochemical evidence for aerobic mineralisation and reductive dechlorination pathways, yet *Dhc* and VC reductive dehalogenase-encoding genes (*vcrA* and *bvcA*) were enriched in both microcosms. The study findings directly influence understanding of VC biodegradation pathways, and constraints on the performance of MNA.

qPCR and RT-qPCR data indicating abundant and active populations of specific microorganisms capable of biodegradation could, with primary lines of evidence for contaminant mass or concentration reduction, support demonstration of MNA. Conversely, qPCR indicating sub-optimal populations or unacclimated populations of microorganisms may help explain why observed rates of biodegradation are lower than expected and the need for intervention.

Threshold values for qPCR data indicating suitable conditions for biodegradation have been cited for a limited number of specific contaminants. For example:

- *Dhc* between 10^4 and 10^6 gene copies per litre can support MNA and $>10^6$ ensures ethene production (Lebrón *et al*., 2015);
- *Dhc* $>10^7$ gene copies per litre are cited as associated with high rates of ethene formation (Lu *et al*., 2006).

Application of this threshold to *Dhc* data is further extended by comparing RT-qPCR data for TCE and VC reductase enzymes (*tceA*, *vcrA* and *bvcA*). VC accumulation is considered likely where *vcrA* and/or *bvcA* concentrations are non-detect or significantly lower than *Dhc* and *tceA*. Where *vcrA* and/or *bvcA* concentrations are similar to *Dhc* and *tceA*, complete dechlorination to ethene is much more likely.

qPCR alone can provide sufficient evidence of biodegradation potential for microorganisms with specific metabolism (e.g. *Dhc*). However, degradation of some contaminants (e.g. 1,4-dioxane) is performed by microorganisms with more varied metabolism. In such cases, RT-qPCR data are required to confirm whether the abundance of these microorganisms is associated with biodegradation of the target contaminant, or abundance of organisms expressing suitable functional genes (Figure A9.2).

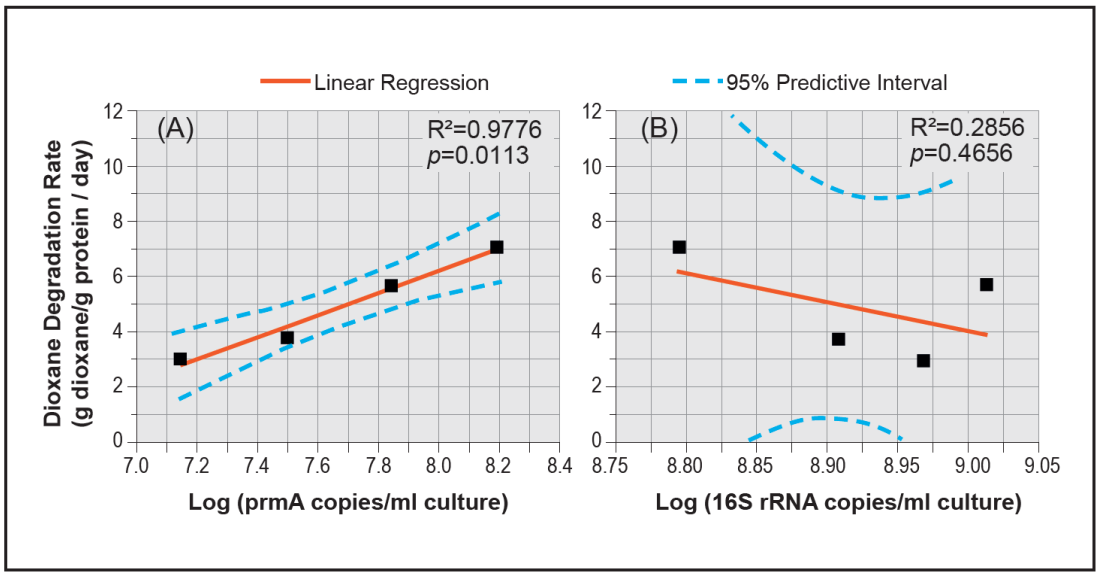


Figure A9.2: Correlation between dioxane degradation rate and abundance of propane monooxygenase alpha subunit (*prmA*) (A) but not 16S rRNA (B) gene copies. Reprinted (adapted) with permission from He *et al*. (2017a). © 2017 American Chemical Society.

A9.2.2 16SrRNA Amplicon Sequencing

16SrRNA amplicon sequencing, so-called "Next Generation Sequencing" (NGS), provides a means to achieve comprehensive microbial community characterisation, insights into community function and dynamics that are simply not possible with qPCR methods that target specific organisms (e.g. Badin *et al*., 2016; He *et al*., 2017b; Toth *et al*., 2021).

NGS provides insights to complex microbial systems, such as those impacted by contaminant mixtures or contaminants for which degradation is performed by consortia rather than key, individual species or groups of microorganisms. NGS can indicate dominant or potential microbial processes, including biodegradation, and assist identifying conditions which may inhibit biodegradation, for example, related to either the site or contaminant matrix. Badin *et al*. (2016) provides intriguing insights to a microbial community and its function following thermal remediation of a PCE source zone. The study demonstrated the enhancement of anaerobic biodegradation of PCE due to release of dissolved organic carbon caused by steam injection and the predominance of abiotic rather than biotic degradation pathways downgradient of the source with combined applications of CSIA and qPCR. The role of the microbiological community to induce abiotic degradation in the plume was evidenced through NGS, that indicated the abundance of iron-reducing, sulfate-reducing bacteria and pyrite (FeS_2) oxidising bacteria, the potential for abiotic degradation with reactive iron sulfide minerals, alongside other lines of evidence.

NGS datasets are typically large and complex, indicating the relative abundance of microbial genus producing 16SrRNA. Novel visualisation techniques facilitate understanding (Figure A9.3) but multivariate statistical methods are typically required to interpret these data. NGS does not report to species or strain-level, nor does it provide information on functional genes. Quantification can be achieved through calibration against qPCR for total 16SrRNA biomass.

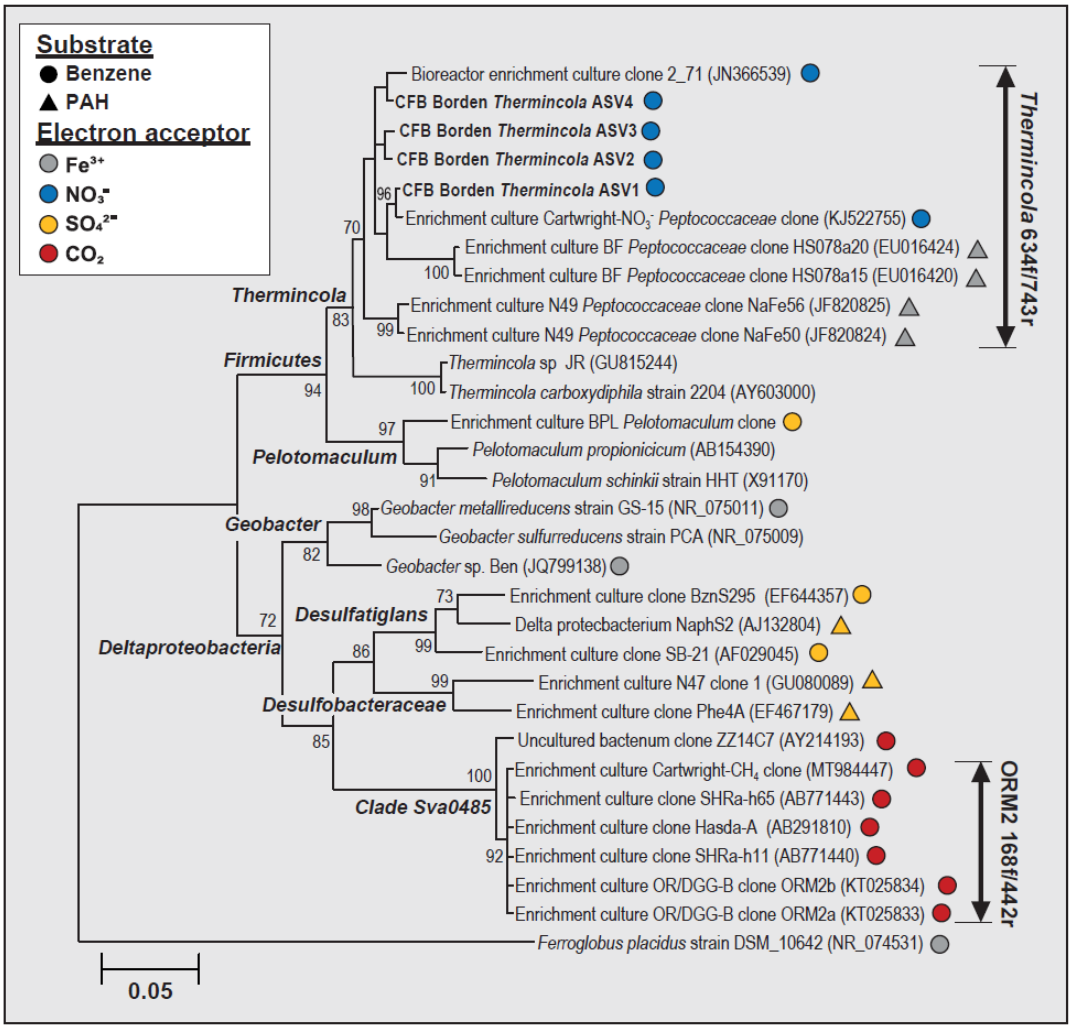


Figure A9.3: Maximum likelihood consensus tree showing the affiliation of near-complete 16SrRNA genes (1231 bp) belonging to anaerobic benzene and PAH-degrading microorganisms, and select reference strains. Additionally, the specificity of the *Thermincola* and ORM2 qPCR primer pairs used in this study is illustrated. Reprinted (adapted) with permission from Toth *et al*. (2021). © 2021 American Chemical Society.

A9.2.3 Metagenome Analysis

Metagenome analysis uses PCR to amplify 4 million base pair genes, including 16SrRNA. So-called "shotgun genomics" provides the most comprehensive characterisation of microbial strains, non-microbial species (e.g. fungi, protozoans), and their functional genes, from which the activity and potential for sustained contaminant degradation can be inferred. Dang *et al*. (2018) applied metagenome sequencing to quantify chlorinated solvent and 1,4-dioxane biodegradation taxonomy and functional genes at five sites. The analysis determined the abundance of (1) genera associated with chlorinated solvent degradation, (2) reductive dehalogenase genes, (3) genes associated with 1,4-dioxane removal, (4) genes associated with aerobic chlorinated solvent degradation, and (5) *Dehalococcoides mccartyi* genes associated with hydrogen and corrinoid metabolism. The work illustrates the importance of metagenome sequencing to provide a more complete picture of the functional abilities of microbial communities and its advantages over simpler MBTs (such as qPCR) because an

unlimited number of functional genes can be quantified. Multivariate statistical methods support a higher level of interpretation that were used to highlight the significant, but typically overlooked, roles of supporting organisms to *Dhc* for anaerobic biodegradation of PCE and TCE, for example (Figure A9.4).

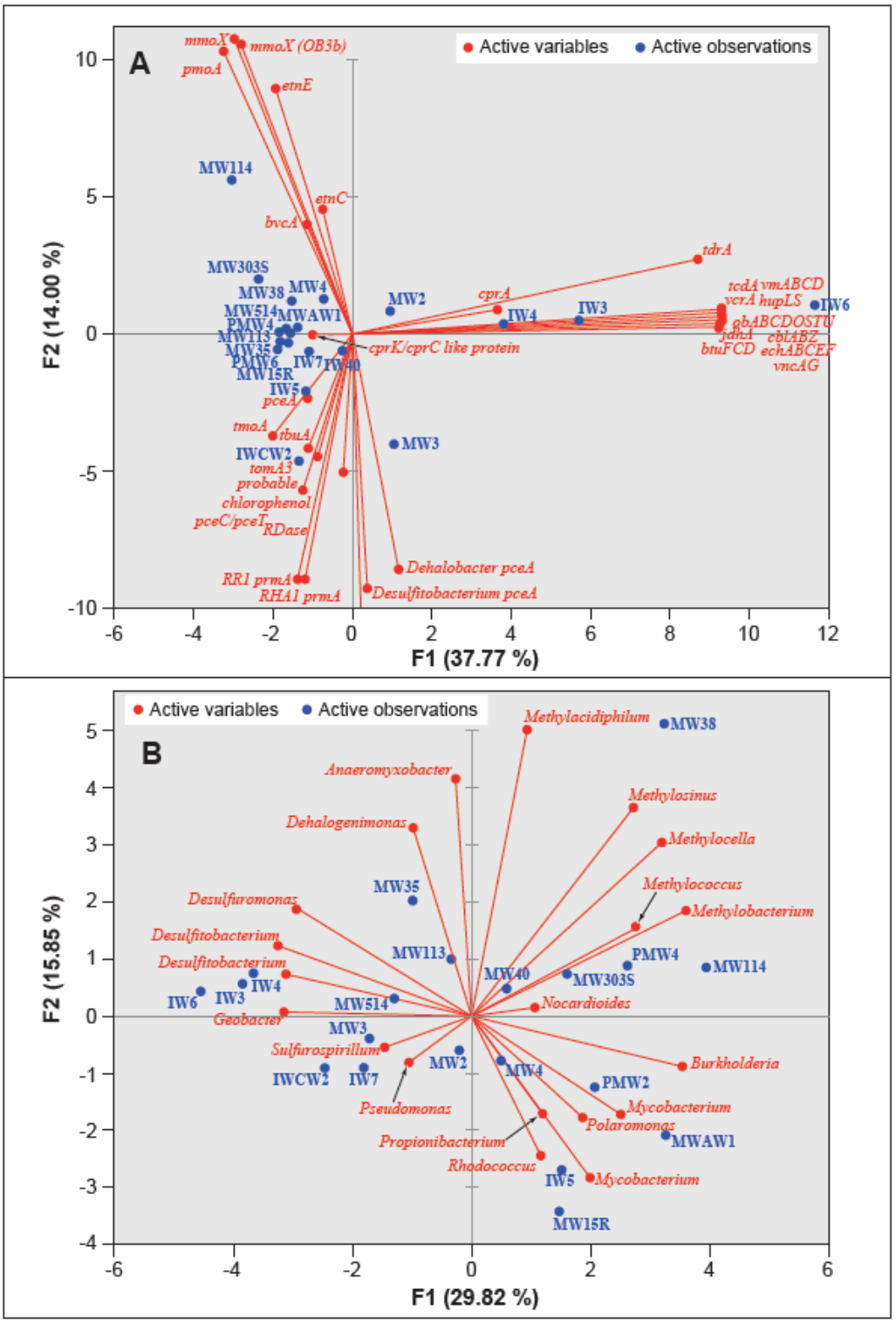


Figure A9.4: Principal component analysis of functional genes (A) and genera (B) associated with chlorinated solvent and 1,4-dioxane biodegradation in groundwater. Reprinted (adapted) with permission from Dang *et al*. (2018). © 2018 American Chemical Society.

A9.3 Sampling for MBTs

Sampling for MBT analysis is not onerous. Commonly, groundwater samples are used for molecular analysis. These can be collected in standard laboratory bottles or by passing a known volume of groundwater through 0.2 µm filters, which are then analysed in the laboratory. Such samples can be therefore collected at the same time as routine groundwater monitoring events to collect data for primary and secondary lines of evidence for MNA. MBTs can also be applied to samples of soil, bedrock or sediment, to characterise biofilms attached to aquifer solids.

Appendix 10: Selected Literature on Natural Attenuation of Key CoPC

Table A10.1: List of selected literature reviews that have critically appraised NA of key CoPC. This list is not exhaustive but is aimed to help the reader identify key review papers.

CoPC	Literature source
Petroleum hydrocarbons	Seagren, E.A. and Becker, J.G., 2002. Review of natural attenuation of BTEX and MTBE in groundwater. Practice Periodical of Hazardous, Toxic, and Radioactive Waste Management. Vol. 6, Issue 3. https://doi.org/10.1061/(ASCE)1090-025X(2002)6:3(156) Thornton, S.F., Morgan, P.M. and Rolfe, S.A., 2016. Bioremediation of hydrocarbons and chlorinated solvents in groundwater: Characterisation, design and performance assessment. In: *Protocols for Hydrocarbon and Lipid Microbiology*. McGenity, T.J., Timmis, K.N. & Nogales, B. (eds), Springer Verlag, Berlin Heidelberg. Series ISSN 1949-2448. pp.11-64, https://doi.org/10.1007/8623_2016_207
Ether oxygenates (petrol additives)	Thornton, S.F., Nicholls, H.C.G., Rolfe, S.A., Mallinson, H.E.H. and Spence, M.J., 2020. Biodegradation and fate of ethyl tert-butyl ether (ETBE) in soil and groundwater: a review. *Journal of Hazardous Materials*. https://doi.org/10.1016/j.jhazmat.2020.122046 Moyer, E.E. and Kostecki, P.T. (eds), 2003. MTBE Remediation Handbook. Springer Publications. ISBN 978-1-4615-0021-6
Biodiesel additives	Thomas, A.O., Leahy, M.C., Smith, J.W.N., Spence, M.J., 2017. The natural attenuation of fatty acid methyl esters (FAME) in soil and groundwater. *Quarterly Journal of Engineering Geology & Hydrogeology*. https://doi.org/10.1144/qjegh2016-130
Chlorinated solvents	Rifai, H.S., Newell, C.J., Wiedemeier, T.H., 2019. Natural attenuation of chlorinated solvents in groundwater. In: Handbook of Solvents, Volume 2: Use, Health and Environment (Third Edition). https://www.sciencedirect.com/book/9781927885413/handbook-of-solvents
Ammonium	Buss, S.R., Morgan, P., Herbert, A., Thornton, S.F., Smith, J.W.N., 2004. A review of ammonium attenuation in soil and groundwater. *Quarterly Journal of Engineering Geology and Hydrogeology*, **37**, 347-359. https://doi.org/10.1144/1470-9236/04-005
Nitrate	Rivett, M.O., Buss, S.R., Morgan, P., Smith, J.W.N. and Bemment, C.D., 2008. Nitrate attenuation in groundwater: A review of biogeochemical controlling processes. *Water Research*, **42**, 4215-4232. https://doi.org/10.1016/j.watres.2008.07.020 Rivett, M.O., Smith, J.W.N., Buss, S.R., Morgan, P., 2007. Nitrate occurrence and attenuation in the major aquifers of England and Wales. *Quarterly Journal of Engineering Geology & Hydrogeology* **40(4)**, 335-352. https://doi.org/10.1144/1470-9236/07-032

CoPC	Literature source
Metals	Gandy, C.J., Smith, J.W.N. and Jarvis, A.P., 2007. Attenuation of mining-derived pollutants in the hyporheic zone: a review. *Science of the Total Environment*, **373**, 435-446. https://doi.org/10.1016/j.scitotenv.2006.11.004
Sulfolane	Dinh, M., Hakimabadi, S.G., Pham, A.L-T., 2020. Treatment of sulfolane in groundwater: A critical review. *J. Env. Management*, **263,** 110385. https://doi.org/10.1016/j.jenvman.2020.110385
Herbicides	Buss, S.R., Thrasher, J., Morgan, P. and Smith, J.W.N., 2006. A review of mecoprop attenuation in the subsurface. *Quarterly Journal of Engineering Geology and Hydrogeology*, **39**, 283-292. https://doi.org/10.1144/1470-9236/04-081
Phenols and cresols	Thornton, S.F., Quigley, S., Spence, M.J., Banwart, S.A., Bottrell, S., Lerner, D.N., 2001. Processes controlling the distribution and natural attenuation of dissolved phenolic compounds in a deep sandstone aquifer. *J. Cont. Hydrol*., 15, 233-267. https://doi.org/10.1016/s0169-7722(01)00168-1

References

Adamson, D.T. and Newell, C.J., 2014. Frequently Asked Questions about Monitored Natural Attenuation in Groundwater. ESTCP Project ER-201211. Environmental Security and Technology Certification Program, Arlington, Virginia. https://www.frtr.gov/matrix/documents/Monitored-Natural-Attenuation/2014-Frequently-Asked-Questions-about-Monitored-Natural-Attenuation-in-Groundwater.pdf

Air Force Center for Environmental Excellence (AFCEE), 2004. Principles and Practices of Enhanced Anaerobic Bioremediation of Chlorinated Solvents. https://www.frtr.gov/matrix/documents/Enhanced-In-Situ-Reductive-Dechlorinated-for-Groundwater/2004-Principles-and-Practices-of-Enhanced-Anaerobic-Bioremediation-of-Chlorinated-Solvents.pdf

Alberti, L., Marchesi, M., Trefiletti, P. and Aravena, R., 2017. Compound-Specific Isotope Analysis (CSIA) Application for Source Apportionment and Natural Attenuation Assessment of Chlorinated Benzenes. Water. 9 (11) 872. https://doi.org/10.3390/w9110872

Alvarez, P.J. and Illman, W.A., 2005. Bioremediation and Natural Attenuation: Process Fundamentals and Mathematical Models. https://doi.org/10.1002/047173862X.ch6

American Society for Testing and Materials (ASTM), 1998. Standard Guide for Remediation of Ground Water by Natural Attenuation at Petroleum Release Sites. ASTM Standard Guide E1943-98. ASTM. https://www.astm.org/e1943-98r15.html

Aronson, D. and Howard, P.H., 1997. Anaerobic Biodegradation of Organic Chemicals in Groundwater: A Summary of Field and Laboratory Studies. https://www.api.org/~/media/files/ehs/clean_water/gw_other/anerobicbiodegrateconstantrpt1998.pdf

Aronson, D., Citra, M., Shuler, K., Printup, H. and Howard, P.H., 1999. Aerobic Biodegradation of Organic Chemicals in Environmental Media: A Summary of Field and Laboratory Studies. https://www.api.org/-/media/files/ehs/clean_water/gw_other/aerobicbiodegrateconstantrpt1999pdf.pdf

Atashgahi, S., Lu, Y., Ramiro-Garcia, J., Peng, P., Maphosa, F., Sipkema, D., Dejonghe, W., Smidt, H. and Springael, D., 2017. Geochemical parameters and reductive dechlorination determine aerobic cometabolic vs aerobic metabolic vinyl chloride biodegradation at oxic/anoxic interface of hyporheic zones. Environ. Sci. Technol. 51 (3), 1626-1634. https://doi.org/10.1021/acs.est.6b05041

Aziz, J.J., Ling, M., Rifai, H., Newell, C.J. and Gonzales, J.R., 2003. MAROS: A Decision Support System for Optimizing Monitoring Plans. 41 (3), 355-367. https://doi.org/10.1111/j.1745-6584.2003.tb02605.x

Aziz, C.E. and Newell, C.J., 2002. BIOCHLOR Natural Attenuation Decision Support System, Version 2.2, March 2002. Groundwater Services, Inc., Houston, Texas.

Aziz, C.E., Newell, C.J., Gonzales, J.R., Haas, P., Clement, T.P. and Sun, Y-W., 2000. BIOCHLOR Version 1.0 User's Manual. EPA/600/R-00/008.

Aziz, J.J., Newell, C.J., Rifai, H.S., Ling, M. and Gonzales J.R., 2000. Monitoring and Remediation Optimization System (MAROS) Software User's Guide.

Badin, A., Broholm, M.M., Jacobsen, C.S., Palau, J., Dennis, P. and Hunkeler, D., 2016. Identification of Abiotic and Biotic Reductive Dechlorination in a Chlorinated Ethene Plume after Thermal Source Remediation by Means of Isotopic and Molecular Biology Tools. Journal of Contaminant Hydrology. 192: 1-19. https://doi.org/10.1016/j.jconhyd.2016.05.003

Ball, W.P. and Roberts P.V., 1991. Long-term sorption of halogenated organic chemicals by aquifer material. 1. Equilibrium. Environmental Science and Technology, 25 (7) pp. 1223-1237. https://doi.org/10.1021/ES00019A002

Barlett, M., Moon, H.S., Peacock, A.A., Hedrick, D.B., Williams, K.H., Long, P.E., Lovley, D. and Jaffe, P.R., 2012. Uranium Reduction and Microbial Community Development in Response to Stimulation with Different Electron Donors. Biodegradation 23, no. 4: 535-46. https://doi.org/10.1007/s10532-011-9531-8

Barnett, B., Townley, L.R., Post, V., Evans, R.E., Hunt, R.J., Peeters, L., Richardson, S., Werner, A.D., Knapton, A. and Boronkay, A., 2012. Australian groundwater modelling guidelines, Waterlines report, National Water Commission, Canberra.

Bedekar, V., Morway, E.D., Langevin, C.D. and Tonkin, M., 2016. MT3D-USGS version 1: A U.S. Geological Survey release of MT3DMS updated with new and expanded transport capabilities for use with MODFLOW: Techniques and Methods 6-A53, 69 p., https://doi.org/10.3133/tm6A53

Beller, H.R., Kane, S.R., Legler, T.C., McKelvie, J.R., Sherwood Lollar, B., Pearson, F., Balser, L. and Mackay, D.M., 2008. Comparative Assessments of Benzene, Toluene, and Xylene Natural Attenuation by Quantitative Polymerase Chain Reaction Analysis of a Catabolic Gene, Signature Metabolites, and Compound-Specific Isotope Analysis'. Environmental Science & Technology. 42, no. 16: 6065–72. https://doi.org/10.1021/es8009666

Bennett, P., 2017. Extending The Applicability of Compound-Specific Isotope Analysis to Low Concentrations of 1,4-Dioxane. SERDP Project ER-2535. Haley & Aldrich, Inc, SERDP.

Boesten, J.J.T.I, Aden, K., Beigel, C., Beulke, S., Dust, M., Dyson, J.S., Fomsgaard, I.S., Jones, R.I., Karlsson, S., A van der Linden, A.M., Richter, O., Magrans, J.O. and Soulas, G., 2006. Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration Sanco/10058/2005, version 2.0. available at: https://esdac.jrc.ec.europa.eu/public_path/projects_data/focus/dk/docs/finalreportFOCDegKinetics.pdf

Bradley, P.M. and Chapelle, F.H., 2010. Chapter 3 - Biodegradation of Chlorinated Ethenes in H.F. Stroo and C.H. Ward (eds.), In Situ Remediation of Chlorinated Solvent Plumes, Springer Science & Business Media, Springer, New York, NY. https://link.springer.com/chapter/10.1007/978-1-4419-1401-9_3

British Standards Institution, 2018. Water quality - Sampling. Preservation and handling of water samples. BS EN ISO 5667-3:2018. https://doi.org/10.3403/30349850

Brown, R.A., Wilson, J.T. and Ferrey, M., 2007. Monitored Natural Attenuation Forum: The Case for Abiotic MNA. Remediation Journal 17 (2) pp 127-137. https://doi.org/10.1002/rem.20128

Buscheck, T.E. and Alcantar, C.M., 1995. Regression Techniques and Analytical Solutions to Demonstrate Intrinsic Bioremediation. In: R.E. Hinchee, J.T. Wilson, and D.C. Downey (Eds.), Intrinsic Bioremediation, pp. 109-116. Battelle Press, Columbus, OH.

Cameron, K., 2004. Better optimization of long-term monitoring networks. Bioremediation Journal 88 (03-04): 89-108. https://doi.org/10.1080/10889860490887464

Carey, G.R., Van Geel. P.J., Wiedemeier, T.H. and McBean, E.A., 2003. A modified radial diagram approach for evaluating natural attenuation trends for chlorinated solvents and inorganic redox indicators. Groundwater monitoring and remediation. 23, 4, 75-84. https://doi.org/10.1111/j.1745-6592.2003.tb00697.x

CL:AIRE, 2008. Principles and practice for the collection of representative groundwater samples. Technical Bulletin 3 (TB3). CL:AIRE. https://www.claire.co.uk/component/phocadownload/category/17-technical-bulletins?download=47:technicalbulletin03

CL:AIRE, 2010a. Sustainable Remediation Forum UK (SuRF-UK): A Framework for Assessing the Sustainability of Soil and Groundwater Remediation. ISBN 978-1-905046-19-5. CL:AIRE, London. https://www.claire.co.uk/component/phocadownload/category/16-surf-uk-bulletins?download=61:surf-uk-framework-final-march-2010

CL:AIRE, 2010b. Insights and Modelling Tools for Designing and Improving Chlorinated Solvent Bioremediation Applications. SABRE Bulletin SAB 4. September 2010. https://www.claire.co.uk/component/phocadownload/category/13-sabre-bulletins?download=196:sabre-bulletin-04

CL:AIRE, 2014. An illustrated handbook of LNAPL transport and fate in the subsurface. CL:AIRE, London. ISBN 978-1-905046-24-9. Download at https://www.claire.co.uk/LNAPL

CL:AIRE, 2019a. An Introduction to Natural Source Zone Depletion at LNAPL Sites. Technical Bulletin 20 (TB20). CL:AIRE. https://www.claire.co.uk/component/phocadownload/category/17-technical-bulletins?download=681:tb-20-an-introduction-to-natural-source-zone-depletion-at-lnapl-sites

CL:AIRE, 2019b. The GroundWater Spatiotemporal Data Analysis Tool (GWSDAT) for Groundwater Quality Analyses. Technical Bulletin 21 (TB21). CL:AIRE. https://www.claire.co.uk/component/phocadownload/category/17-technical-bulletins?download=682:tb-21-the-groundwater-spatiotemporal-data-analysis-tool-gwsdat-for-groundwater-quality-analyses-2019

CL:AIRE, 2022. Resilience and Adaptation for Sustainable Remediation. SuRF-UK Bulletin SuRF 5. CL:AIRE. https://www.claire.co.uk/component/phocadownload/category/16-surf-uk-bulletins?download=890:surf-5-bulletin-resilience-2022

CL:AIRE, 2024. Guidance on Natural Source Zone Depletion. CL:AIRE, Reading. ISBN 978-1-905046-44-7. Download at https://www.claire.co.uk/NSZD

Clark, K., Taggart, D.M., Baldwin, B.R., Ritalahti, K.M., Murdoch, R.W., Hatt, J.K. and Löffler, F.E., 2018. Normalized Quantitative PCR Measurements as Predictors for Ethene Formation at Sites Impacted with Chlorinated Ethenes. Environmental Science & Technology 52, no. 22: 13410-20. https://doi.org/10.1021/acs.est.8b04373

Clement, T.P., 1997. RT3D - A Modular Computer Code for Simulating Reactive Multi-Species Transport in 3-Dimensional Groundwater Aquifers. Pacific Northwest National Laboratory, Richland, WA, USA. PNNL-11720. https://www.pnnl.gov/sites/default/files/media/file/PNNL_11720_RT3Dv1_Manual.pdf

Cooperative Research Centre for Contamination Assessment and Remediation of the Environment (crcCARE), 2010. A technical guide for demonstrating monitored natural attenuation of petroleum hydrocarbons in groundwater. crcCARE Technical report 15. https://www.crccare.com/files/dmfile/CRCCARETechReport15-Atechnicalguidefordemonstratingmonitorednaturalattenuationofpetroleumhydrocarbonsingroundwater2.pdf

Cui, G., Lartey-Young, G., Chen, C., Ma, L., 2021. Photodegradation of pesticides using compound-specific isotope analysis (CSIA): a review. RSC Adv. 11. pp 25122-25140. https://doi.org/10.1039/D1RA01658J

Dang, H., Kanitkar, Y.H., Stedtfeld, R.D., Hatzinger, P.B., Hashsham, S.A. and Cupples, A.M., 2018. Abundance of Chlorinated Solvent and 1,4-Dioxane Degrading Microorganisms at Five Chlorinated Solvent Contaminated Sites Determined via Shotgun Sequencing. Environmental Science & Technology 52, no. 23: 13914-24. https://doi.org/10.1021/acs.est.8b04895

Doherty, J., 2015. Calibration and Uncertainty Analysis for Complex Environmental Models. Watermark Numerical Computing, Brisbane, Australia.

Doherty, J.E. and Hunt, R.J., 2010. Approaches to highly parameterized inversion—A guide to using PEST for groundwater-model calibration: U.S. Geological Survey Scientific Investigations Report 2010-5169, 59 p. https://pubs.usgs.gov/sir/2010/5169/pdf/GWPEST_sir2010-5169.pdf

Doherty, J.E., Fienen, M.N. and Hunt, R.J., 2010a. Approaches to highly parameterized inversion: Pilot-point theory, guidelines, and research directions: U.S. Geological Survey Scientific Investigations Report 2010-5168, 36 p. https://pubs.usgs.gov/sir/2010/5168/

Doherty, J.E., Hunt, R.J. and Tonkin, M.J., 2010b. Approaches to highly parameterized inversion: A guide to using PEST for model-parameter and predictive-uncertainty analysis: U.S. Geological Survey Scientific Investigations Report 2010-5211, 71 p. https://pubs.usgs.gov/sir/2010/5211/pdf/uncpest_sir2010-5211.pdf

Dragun, J., 1988. The Soil Chemistry of Hazardous Materials. Hazardous Materials Control Research Institute, Silver Spring, Maryland.

Elsner, M., McKelvie, J., Lacrampe Couloume, G. and Sherwood Lollar, B., 2007. Insight into Methyl Tert-Butyl Ether (MTBE) Stable Isotope Fractionation from Abiotic Reference Experiments. Environmental Science & Technology 2, 41 (16), 5693-5700. https://doi.org/10.1021/es070531o

Environment Agency, 2000. Guidance on the assessment and monitoring of natural attenuation of contaminants in groundwater. R&D Publication 95. Environment Agency, Bristol. ISBN 1 857 05263 2.

Environment Agency, 2006. Remedial Targets Methodology: Hydrogeological Risk Assessment for Land Contamination, Environment Agency. https://www.gov.uk/government/publications/remedial-targets-worksheet-v22a-user-manual

Environment Agency, 2023. Land Contamination: Risk Management. https://www.gov.uk/government/publications/land-contamination-risk-management-lcrm

Falta, R.W., 2008. Methodology for comparing source and plume remediation alternatives. Groundwater 46: 272–285. https://doi.org/10.1111/j.1745-6584.2007.00416.x

Farhat, S.K., de Blanc, P.C., Newell, C.J. and Gonzales, J.R., 2011. Source DK Remediation Time Frame Decision Support System, User's Manual, Air Force Center for Environmental Excellence, Technology Transfer Division.

Farhat, S.K., Newell, C.J., Falta, R.W. and Lynch, K., 2018. REMChlor-MD toolkit user's manual. ER-201426.

Farhat, S.K., Newell, C.J. and Nichols, E.M., 2006. Mass Flux Toolkit to Evaluate Groundwater Impacts, Attenuation, and Remediation Alternatives. User's Manual.

Farhat, S.K., Newell, C.J., Sale, T.C., Dandy, D.S., Wahlberg, J.J., Seyedabbasi, M.A., McDade, J.M. and Mahler, N.T., 2012. Matrix Diffusion Toolkit, developed for the Environmental Security Technology Certification Program (ESTCP) by GSI Environmental Inc., Houston, Texas. https://www.gsi-net.com/en/software/free-software/matrix-diffusion-toolkit/matrix-diffusion-toolkit-users-manual-version-1-23/file.html

Fischer, A., Herklotz, I., Herrmann, S., Thullner, M., Weelink, S.A.B., Stams, A.J.M., Schlömann, M., Richnow, H-H. and Vogt, C., 2008. Combined carbon and hydrogen isotope fractionation investigations for elucidating benzene biodegradation pathways. Environmental Science and Technology 42: 4356-4363. https://doi.org/10.1021/es702468f

Freeze, R.A. and Cherry, J.A. 1979. Groundwater. Prentice-Hall: New Jersey. Available online: http://hydrogeologistswithoutborders.org/wordpress/1979-english/.

Garg, S., Newell, C.J., Kulkarni, P.R., King, D.C., Adamson, D.T., Renno, M.I. and Sale, T., 2017. Overview of Natural Source Zone Depletion: Processes, Controlling Factors, and Composition Change. Groundwater Monit R 37, 62-81. https://doi.org/10.1111/gwmr.12219.

Golder, 2018. ConSim. www.consim.co.uk. Last updated in 2018. Last accessed 17 July 2020.

Gray, J.R., Lacrampe-Couloume, G., Gandhi, D., Scow, K.M., Wilson, R.D., Mackay, D.M. and Sherwood Lollar, B., 2002. Carbon and hydrogen isotopic fractionation during biodegradation of methyl tert-butyl ether. Environmental Science and Technology 36: 1931-1938. https://doi.org/10.1021/ES011135N

Groundwater Services International (GSI), 2006. Mass Flux Toolkit, User's Manual. Version 1.0 March, Groundwater Services, Inc.

GSI, 2012. Mann Kendall Toolkit User's Manual, Groundwater Services International.

Guerrero, J.S.P. and Skaggs, T.H., 2010. Analytical solution for one-dimensional advection–dispersion transport equation with distance-dependent coefficient. Journal of Hydrology. 390. pp. 57-65. https://doi.org/10.1016/j.jhydrol.2010.06.030

Harbaugh, A.W., 2005. MODFLOW-2005, The U.S. Geological Survey Modular Ground-Water Model—The Ground-Water Flow Process. Reston, Virginia: USGS Techniques and Methods 6-A16.

Harbaugh, A.W., Banta, E.R., Hill, M.C. and McDonald, M.G., 2000. MODFLOW-2000, The U.S. Geological Survey modular ground-water model—User guide to modularization concepts and the ground-water flow process: USGS Open-File Report 00-92. Reston, Virginia: USGS.

He, Y., Su, C., Wilson, J., Wilkin, R., Adair, C., Lee, T., Bradley, P. and Ferrey, M., 2009. Identification and characterization methods for reactive minerals responsible for natural attenuation of chlorinated organic compounds in ground water. EPA Report EPA 600/R-09/115.

He, Y.T., Wilson, J.T., Su, C. and Wilkin, R.T., 2015. Review of abiotic degradation of chlorinated solvents by reactive iron minerals in aquifers. Groundwater Monitoring & Remediation 35, no. 3: pp. 57-75. https://doi.org/10.1111/gwmr.12111

He, Y., Mathieu, J., Yang, Y., Yu, P., da Silva, M.L.B. and Alvarez, P.J.J., 2017a. 1,4-Dioxane Biodegradation by Mycobacterium dioxanotrophicus PH-06 is Associated with a Group-6 Soluble Di-Iron Monooxygenase. Environ. Sci. Technol. Lett. 4, 494-499. https://doi.org/10.1021/acs.estlett.7b00456

He, Y., Wei, K., Si, K., Mathieu, J., Li, M., Alvarez, P.J.J., 2017b. Whole-Genome Sequence of the 1,4-Dioxane-Degrading Bacterium Mycobacterium dioxanotrophicus PH-06. Genome Announcements 5, e00625-17. https://doi.org/10.1128/genomeA.00625-17

Höhener, P., 2016. Simulating stable carbon and chlorine isotope ratios in dissolved chlorinated groundwater pollutants with BIOCHLOR-ISO. Journal of Contaminant Hydrology, volume 195, pp. 52-61. https://doi.org/10.1016/j.jconhyd.2016.11.002

Höhener, P., Li, Z.M., Julien, M., Nun, P., Robins, R.J. and Remaud, G.S., 2017. Simulating Stable Isotope Ratios in Plumes of Groundwater Pollutants with BIOSCREEN-AT-ISO. Groundwater, Volume 55 (2). https://doi.org/10.1111/gwat.12472

Honeyman, B.D., 1999. Colloidal culprits in contamination. Nature 397. pp. 23-24. https://doi.org/10.1038/16150

Hunt, R.J., Doherty, J. and Tonkin, M.J., 2007. Are models too simple? Arguments for increased parameterization. Groundwater, 45, no. 3, 254-262. https://doi.org/10.1111/j.1745-6584.2007.00316.x

Hyun, S.P., Davis, J.A., Sun, K., Hayes, K.F., 2012. Uranium (VI) Reduction by Iron (II) Monosulfide Mackinawite. Environmental Science & Technology, 46(6): 3369-3376. https://doi.org/10.1021/es203786p

Illman, W.A. and Alvarez, P.J., 2009. Performance Assessment of Bioremediation and Natural Attenuation, Critical Reviews in Environmental Science and Technology, 39:4, 209-270. https://doi.org/10.1080/10643380701413385

Interstate Technology & Regulatory Council (ITRC), 2010. Use and Measurement of Mass Flux and Mass Discharge. Technology Overview. The Interstate Technology & Regulatory Council Integrated DNAPL Site Strategy Team. MASS FLUX 1. https://itrcweb.org/teams/projects/mass-flux

ITRC, 2011. Environmental Molecular Diagnostics Fact Sheets. EMD-1. Washington, D.C.: Interstate Technology & Regulatory Council, Environmental Molecular Diagnostics Team. https://itrcweb.org/teams/projects/environmental-molecular-diagnostics

ITRC, 2013. Groundwater Statistics and Monitoring Compliance, Statistical Tools for the Project Life Cycle. GSMC-1. Washington, D.C. Interstate Technology & Regulatory Council, Groundwater Statistics and Monitoring Compliance Team. http://www.itrcweb.org/gsmc-1/

Jeffers, P.M., Ward, L.M., Woytowitch, L.M. and Wolfe, N.L., 1989. Homogeneous hydrolysis rate constants for selected chlorinated methanes, ethanes, ethenes, and propanes. Environmental Science and Technology, v. 23, no. 8, p. 965-969. https://doi.org/10.1021/ES00066A006

Jones, W.R., Spence, M.J., Bowman, A.W., Evers, L. and Molinari, D.A., 2014. A software tool for the spatiotemporal analysis and reporting of groundwater monitoring data. Environmental Modelling & Software, 55 (2014) 242-249. https://doi.org/10.1016/j.envsoft.2014.01.020

Konikow, L.F., 2011. The Secret to Successful Solute-Transport Modeling. Groundwater, 49: 144-159. https://doi.org/10.1111/j.1745-6584.2010.00764

Kuder, T. and Philp, P., 2008. Modern Geochemical and Molecular Tools for Monitoring In-Situ Biodegradation of MTBE and TBA. Reviews in Environmental Science and Bio/Technology 7, no. 1: 79-91. https://doi.org/10.1007/s11157-007-9123-6

Kuder, T., Philp, P., van Breukelen, B., Thouement, H., Vanderford, M. and Newell, C., 2014. Integrating Stable Isotope Analysis and Reactive Transport Modeling for Assessing Chlorinated Solvent Degradation. Users Guide. ESTCP Project ER-201029. Environmental Security and Technology Certification Program, Arlington, Virginia.

Kueper, B., Wealthall, G., Smith, J., Leharne, S. and Lerner, D., 2003. An illustrated handbook of DNAPL transport and fate in the subsurface. Environment Agency, Bristol. ISBN 1 844 32066 9.

Kulkarni, P.R., Newell, C.J., Krebs, C.J., Britt, S. and McHugh, T.E., 2015. Methods for Minimization and Management of Variability in Long-Term Groundwater Monitoring Results, ESTCP Project ER-201209.

Lebrón, C.A., Wiedemeier, T.H., Wilson, J.T., Löffler, F.E., Hinchee, R.E. and Singletary, M.A., 2015. Development and Validation of a Quantitative Framework and Management Expectation Tool for the Selection of Bioremediation Approaches (Monitored Natural Attenuation [MNA], Biostimulation and/or Bioaugmentation) at Chlorinated Solvent Sites. ER-201129.

Lee, W. and Batchelor, B., 2002. Reductive Capacity of Natural Reductants. Environmental Science & Technology, 37(3): 535-541. https://doi.org/10.1021/es025830m

Lelliott, M. and Wealthall, G., 2004. Qualitative, quantitative, and visual methods to assess natural attenuation. British Geological Survey Internal Report, IR/04/169.

Liang, H., Falta, R.W., Newell, C.J., Farhat, S.K., Suresh, P., Rao, C. and Basu, N., 2010. Decision & Management Tools for DNAPL Sites: Optimization of Chlorinated Solvent Source and Plume Remediation Considering Uncertainty. ESTCP Project ER-200704.

Liang, X., Dong, Y., Kuder, T., Krumholz, L.R., Philp, R.P. and Butler, E.C., 2007. Distinguishing abiotic and biotic transformation of tetrachloroethylene and trichloroethylene by stable carbon isotope fractionation. Environmental Science and Technology 41: 7094-7100. https://doi.org/10.1021/ES070970N

Liang, Y., Martinez, A., Hornbuckle, K.C. and Mattes, T.E., 2014. Potential for Polychlorinated Biphenyl Biodegradation in Sediments from Indiana Harbor and Ship Canal. International Biodeterioration & Biodegradation 89: 50-57. https://doi.org/10.1016/j.ibiod.2014.01.005

Lovanh, N., Zhang, Y-K., Heathcote, R.C. and Alvarez, P.J.J., 2000. Guidelines to Determine Site-Specific Parameters for Modeling the Fate and Transport of Monoaromatic Hydrocarbons in Groundwater. The University of Iowa. https://www.iowadnr.gov/portals/idnr/uploads/ust/monohydrocarbonsguide.pdf

Lu, X., Wilson, J.T. and Kampbell, D.H., 2006. Relationship between Dehalococcoides DNA in Ground water and Rates of Reductive Dechlorination at Field Scale. Water Research 40: 3131-3140. https://doi.org/10.1016/j.watres.2006.05.030

Mabey, W.R. and Mill T., 1978. Critical review of hydrolysis of organic compounds in water under environmental conditions: Journal of Physical and Chemical Reference Data, v. 7, no. 2, p. 383-415. https://doi.org/10.1063/1.555572

Mattes, T.E., Alexander, A.K. and Coleman, N.V., 2010. Aerobic biodegradation of the chloroethenes: pathways, enzymes, ecology, and evolution. FEMS Microbiology (34) pp. 445-475. https://doi.org/10.1111/j.1574-6976.2010.00210.x

McDonald, M.G. and Harbaugh, A.W., 1988. A modular three- dimensional finite-difference ground-water flow model: U.S. Geological Survey Techniques of Water-Resources Investigations, book 6, chap. A1, 586 p.

McHugh, T.E., 2015. Monitoring Frequency Optimization Toolkit User's Guide. ESTCP Project ER-201209.

McHugh, T.E., Beckley, L.M., Liu, C.Y. and Newell, C.J., 2011. Factors Influencing Variability in Groundwater Monitoring Data Sets. Groundwater Monitoring & Remediation, 31 (2), 92-101. https://doi.org/10.1111/j.1745-6592.2011.01337.x

McHugh, T.E., Kulkarni, P.R., Beckley, L.M., Newell, C.J. and Strasters, B., 2015. A New Method to Optimize Monitoring Frequency and Evaluate Long-Term Concentration Trends, Task 2 & 3 Report, Methods for Minimization and Management of Variability in Long-Term Groundwater Monitoring Results, ESTCP Project ER-201209.

McLean, M.I., 2018. Spatio-temporal models for the analysis and optimisation of groundwater quality monitoring networks. PhD thesis, University of Glasgow.

McMahon, A., Heathcote, J., Carey, M. and Erksine, A., 2001a. Guidance on the Assessment and Interrogation of Subsurface Analytical Contaminant Fate and Transport Models. National Groundwater & Contaminated Land Centre report NC/99/38/1. Environment Agency, Bristol. ISBN 1 857 05486 5.

McMahon, A., Heathcote, J., Carey, M. and Erksine, A., 2001b. Guide to Good Practice for the Development of Conceptual Models and the Selection and Application of Mathematical Models of Contaminant Transport Processes in the Subsurface. National Groundwater & Contaminated Land Centre report NC/99/38/2. Environment Agency, Bristol. ISBN 1 857 05610 8.

McMahon, A., Heathcote, J., Carey, M., Erksine, A. and Barker, J., 2001c. Guidance on Assigning Values to Uncertain Parameters in Subsurface Contaminant Fate and Transport Modelling. National Groundwater & Contaminated Land Centre report NC/99/38/3. Environment Agency, Bristol. ISBN 1 857 05605 1.

Meckenstock, R.U., Elsner, M., Griebler, C., Lueders, T., Stumpp, C., Aamand, J., Agathos, S.N., Albrechtsen, H-J., Bastiaens, L., Bjerg, P.L., Boon, N., Dejonghe, W., Huang., W.E., Schmidt, S.I., Smolders, E., Sørensen, S.R., Springael, D. and van Breukelen, B.M., 2015. Biodegradation: Updating the Concepts of Control for Microbial Cleanup in Contaminated Aquifers. Environmental Science & Technology, 49, 7073-7081. https://doi.org/10.1021/acs.est.5b00715

Mendez. E., Widdowson, M., Brauner, S., Chapelle, F. and Casey, C., 2004. Natural attenuation software (NAS): A computer program for estimating remediation times of contaminated groundwater, in Latini, G., Passerini, G., and Brebbia, C., eds., Development and Application of Computer Techniques to Environmental Studies X: WIT Press, p. 185-194.

Mikkonen, A., Yläranta, K., Tiirola, M., Ambrosio, L., Dutra, L., Salmi, P., Romantschuk, M., Copley, S., Ikäheimo, J. and Sinkkonen, A., 2018. Successful Aerobic Bioremediation of Groundwater Contaminated with Higher Chlorinated Phenols by Indigenous Degrader Bacteria. Water Research 138: 118-28. https://doi.org/10.1016/j.watres.2018.03.033

NAFTA Technical Working Group on Pesticides, 2015. Guidance for Evaluating and Calculating Degradation Kinetics in Environmental Media. https://www.epa.gov/sites/production/files/2015-09/documents/degradation-kin.pdf

Naval Facilities Engineering Systems Command (NAVFAC), 2021. FACT SHEET Environmental Molecular Diagnostics (EMDs): Molecular Biology-Based Tools.

New Jersey Department of Environmental Protection (NJDEP), 2012. Monitored Natural Attenuation Technical Guidance, New Jersey Department of Environmental Protection, Site Remediation Program, https://clu-in.org/download/techfocus/na/mna_NJ_guid_2012.pdf

Newell, C., McLeod, R.K. and Gonzales, J.R., 1996. BIOSCREEN Natural Attenuation Decision Support System, User's Manual, Version 1.3 EPA/600/R-96/087. Washington, DC: EPA Office of Research and Development.

Newell, C.J., Rifai, H.S., Wilson, J.T., Connor, J.A., Aziz, J.A. and Suarez, M.P., 2002. Calculation and use of first-order rate constants for monitored natural attenuation studies, report no. EPA/540/S-02/500, USEPA, Cincinnati, Ohio.

Newell, C.J., Adamson, D.T., Kulkarni, P.R., Nzeribe, B.N., Connor, J.A., Popovic, J. and Stroo, H.F., 2021. Monitored Natural Attenuation to Manage PFAS Impacts to Groundwater: Scientific Basis, Groundwater Monitoring & Remediation 41, 4, 76-89. https://doi.org/10.1111/gwmr.12486

NICOLE, 2005. Monitored Natural Attenuation: Demonstration and review of the applicability of MNA at 8 field sites. Part 1: Main Report.

Oka, A.R., Phelps, C.D., Zhu, X., Saber, D.L. and Young,. L.Y., 2011. Dual Biomarkers of Anaerobic Hydrocarbon Degradation in Historically Contaminated Groundwater. Environmental Science & Technology 45, no. 8: 3407-14. https://doi.org/10.1021/es103859t

Ottosen, C.B., Bjerg, P.L., Hunkeler, D., Zimmermann, J., Tuxen, N., Harrekilde, D., Bennedsen, L., Leonard, G., Brabæk, L., Kristensen, I.L. and Broholm, M.M., 2021. Assessment of chlorinated ethenes degradation after field scale injection of activated carbon and bioamendments: Application of isotopic and microbial analyses. Journal of Contaminant Hydrology 240, 103794. https://doi.org/10.1016/j.jconhyd.2021.103794

Ottosen, C.B., Murray, A.M., Broholm, M.M. and Bjerg, P.L., 2019. In Situ Quantification of Degradation Is Needed for Reliable Risk Assessments and Site-Specific Monitored Natural Attenuation, Environ. Sci. Technol. 2019, 53, 1-3. https://doi.org/10.1021/acs.est.8b06630

Ottosen, C.B., Rønde, V., McKnight, U.S., Annable, M.D., Broholm, M.M., Devlin, J.F. and Bjerg, P.L., 2020. Natural attenuation of a chlorinated ethene plume discharging to a stream: Integrated assessment of hydrogeological, chemical and microbial interactions. Water Research 186, 116332. https://doi.org/10.1016/j.watres.2020.116332

Parker, B.L., Gillham, R.W. and Cherry, J.A., 1994. Diffusive disappearance of immiscible-phase organic liquids in fractured geologic media. Groundwater, 32 (5) pp. 805-820. https://doi.org/10.1111/j.1745-6584.1994.tb00922.x

Parkhurst, D.L. and Appelo, C.A.J.,1999. User's guide to PHREEQC (version 2) – a computer program for speciation, batch-reaction, one-dimensional transport, and inverse geochemical calculations. Water-Resources Investigations Report 99-4259. Denver, Colorado 1999.

Prommer, H. and Post, V., 2010. PHT3D: A reactive multicomponent transport model for saturated porous media.

Puigserver, D., Herrero, J., Parker, B.L. and Carmona, J.M., 2020. Natural Attenuation of Pools and Plumes of Carbon Tetrachloride and Chloroform in the Transition Zone to Bottom Aquitards and the Microorganisms Involved in Their Degradation. Science of The Total Environment 712: 135679. https://doi.org/10.1016/j.scitotenv.2019.135679

Qiao, W., Luo, F., Lomheim, L., Mack, E.E., Ye, S., Wu, J. and Edwards, E.A., 2018. A Dehalogenimonas Population Respires 1,2,4-Trichlorobenzene and Dichlorobenzenes. Environmental Science & Technology 52, no. 22: 13391-98. https://doi.org/10.1021/acs.est.8b04239

Ramos García, A.A., Adamson, D.T., Wilson, J.T., Lebrón, C., Danko, A.S. and Freedman, D.L., 2022. Evaluation of natural attenuation of 1,4-dioxane in groundwater using a ^{14}C assay, Journal of Hazardous Materials, Volume 424, Part C. https://doi.org/10.1016/j.jhazmat.2021.127540

Reilly, T.E. and Harbaugh, A.W., 2004. Guidelines for evaluating ground-water flow models: U.S. Geological Survey Scientific Investigations Report 2004-5038, 30 p.

Renard, P., 2007. Stochastic Hydrogeology: What Professionals Really Need? Groundwater 45, No. 5, pp 531-541. https://doi.org/10.1111/j.1745-6584.2007.00340.x

Ricker, J.A., 2008. A Practical Method to Evaluate Ground Water Contaminant Plume Stability, Groundwater Monitoring & Remediation, 28, 4, 85-94, https://doi.org/10.1111/j.1745-6592.2008.00215.x

Rivett, M.O., Roche, R.S., Tellam, J.H. and Herbert, A.W., 2019. Increased organic contaminant residence times in the urban riverbed due to the presence of highly sorbing sediments of the Anthropocene. Journal of Hydrology X, 3: 100023 https://doi.org/10.1016/j.hydroa.2019.100023

Rivett, M.O. and Thornton, S.F., 2008, Monitored natural attenuation of organic contaminants in groundwater: principles and application, Proceedings of the Institution of Civil Engineers, Water Management 161, December 2008 Issue WM6, pp. 381-392, https://doi.org/10.1680/wama.2008.161.6.381

Scheutz, C., Durant, N.D., Hansen, M.H. and Bjerg, P.L., 2011. Natural and Enhanced Anaerobic Degradation of 1,1,1-Trichloroethane and Its Degradation Products in the Subsurface – A Critical Review. Water Research 45, no. 9: 2701-23. https://doi.org/10.1016/j.watres.2011.02.027

Thornton, S.F., 2019, Natural Attenuation of Hydrocarbon Compounds in Groundwater. In: Steffan R. (eds) Consequences of Microbial Interactions with Hydrocarbons, Oils, and Lipids: Biodegradation and Bioremediation. Handbook of Hydrocarbon and Lipid Microbiology. Springer, Cham. https://doi.org/10.1007/978-3-319-50433-9_3

Thornton, S.F., Bottrell, S.H. Pickup, R.P. Spence, M.J. and Spence, K.H., 2006. Processes controlling the natural attenuation of fuel hydrocarbons and MTBE in the UK Chalk aquifer. CL:AIRE RP03 Research Project Report, pp.96 https://www.claire.co.uk/information-centre/cl-aire-publications?start=9

Thornton, S.F., Davison, R.M., Lerner, D.N. and Banwart, S.A., 1998. Electron Balances in Field Studies of Intrinsic Bioremediation. In: GQ98: Groundwater Quality: Remediation and Protection, Tübingen, Germany, Sept 1998. IAHS Publication No 250, 273-282.

Thornton, S.F., Lerner, D.N. and Banwart, S.A., 2001. Assessing the natural attenuation of organic contaminants in aquifers using plume-scale electron and carbon balances: Model development with analysis of uncertainty and parameter sensitivity, J. of Contaminant Hydrology, 53, 199-232. https://doi.org/10.1016/s0169-7722(01)00167-x

Thornton, S.F., Morgan, P.M. and Rolfe, S.A., 2016. Bioremediation of hydrocarbons and chlorinated solvents in groundwater: Characterisation, design and performance assessment. In : Protocols for Hydrocarbon and Lipid Microbiology. McGenity, T.J., Timmis, K.N. & Nogales, B. (eds), Springer-Verlag, Berlin Heidelberg. Series ISSN 1949-2448. pp.11-64. https://doi.org/10.1007/8623_2016_207

Torres, E., 2019. Groundwater Monitoring Program Optimization – A Multifaceted Technical Approach. Geosyntec Consultants. White Paper, unpublished.

Toth, C.R.A., Luo, F., Bawa, N., Webb, J., Guo, S., Dworatzek, S. and Edwards, E.A., 2021. Anaerobic Benzene Biodegradation Linked to Growth of Highly Specific Bacterial Clades (preprint). Microbiology. https://doi.org/10.1101/2021.01.23.427911

United Kingdom Technical Advisory Group (UK TAG), 2012, Groundwater Trend Assessment, UK Technical Advisory Group on the Water Framework Directive.

USEPA, 2008. A Guide for Assessing Biodegradation and Source Identification of Organic Ground Water Contaminants using Compound Specific Isotope Analysis (CSIA).

USEPA, 2021. Characterisation and Monitoring. Contaminated Site Clean-up Information. https://clu-in.org/characterization/technologies/hrsc/hrscintro.cfm

Wang, G., Allen-King, R.M., Choung, S., Feenstra, S., Watson, R. and Kominek, M., 2013. A practical measurement strategy to estimate nonlinear chlorinated solvent sorption in low f_{oc} sediments. Groundwater Monit. Remed., 33(1), 87-96. https://doi.org/10.1111/j.1745-6592.2012.01413.x

Washington State Department of Ecology, 2005. Guidance on Remediation of Petroleum-Contaminated Ground Water by Natural Attenuation. Publication No. 05-09-091 (version 1.0).

Weatherill, J.J., Atashgahi, S., Schneidewind, U., Krause, S., Ullah, S., Cassidy, N., Rivett, M.O., 2018. Natural attenuation of chlorinated ethenes in hyporheic zones: A review of key biogeochemical processes and in-situ transformation potential. Water Research 128, 362-382. https://doi.org/10.1016/j.watres.2017.10.059

Wiedemeier, T.H., Rifai, H.S., Newell, C.J., Wilson, J.T., 1999. Natural Attenuation of Fuels and Chlorinated Solvents in the Subsurface. John Wiley & Sons, Inc.: Canada.

Wiedemeier, T.H., Swanson, M.A., Moutou, D.F., Gordon, E.K., Wilson, J.T., Wilson, B.H., Kampbell, D.H., Haas, P.E., Miller, R.N., Hansen, J.E. and Chappelle, F.H., 1998. Technical Protocol for Evaluating Natural Attenuation of Chlorinated Solvents in Groundwater. National Risk Management Research Laboratory, Office of Research and Development, United States Environmental Protection Agency (USEPA).

Wiedemeier, T.H., Wilson, B.H., Ferrey, M.L. and Wilson, J.T., 2017. Efficacy of an In-Well Sonde to Determine Magnetic Susceptibility of Aquifer Sediment. Groundwater Monit R, 37: 25-34. https://doi.org/10.1111/gwmr.12197

Wilson. J.T., 2011. An Approach for Evaluating the Progress of Natural Attenuation in Groundwater. National Risk Management Research Laboratory, Office of Research and Development, United States Environmental Protection Agency (USEPA).

Wilson, R.D., Thornton S.F., Hüttmann, A., Gutierrez-Neri, M. and Slenders, H., 2005. CoronaScreen: process-based models for Natural attenuation assessment. Guidance for the application of NA assessment screening models. CORONA project. Contract number EVK1-CT-2001-00087.

Wilson, R.D., Thornton, S.F. and Mackay, D.M., 2004. Challenges in monitoring the natural attenuation of spatially variable plumes. Biodegradation, 15, 359-369. https://doi.org/10.1023/B:BIOD.0000044591.45542.a9

Xiao, Z., Jiang, W., Chen, D. and Xu, Y., 2020. Bioremediation of typical chlorinated hydrocarbons by microbial reductive dechlorination and its key players: A review. Ecotoxicology and Environmental Safety, 202 (1). https://doi.org/10.1016/j.ecoenv.2020.110925

Yan, J., Rash, B.A., Rainey, F.A. and Moe, W.M., 2009. Isolation of Novel Bacteria within the Chloroflexi Capable of Reductive Dechlorination of 1,2,3-Trichloropropane. Environmental Microbiology 11, no. 4: 833–43. https://doi.org/10.1111/j.1462-2920.2008.01804.x

Zheng, C., 1990. MT3D, A modular three-dimensional transport model for simulation of advection, dispersion and chemical reactions of contaminants in groundwater systems, Report to the U.S. Environmental Protection Agency, 170 p.

Zheng, C. and Wang P.P., 1999. MT3DMS, A modular three-dimensional multi-species transport model for simulation of advection, dispersion and chemical reactions of contaminants in groundwater systems; documentation and user's guide, U.S. Army Engineer Research and Development Center Contract Report.

Zimmermann, J., Halloran, L.J.S. and Hunkeler, D., 2020. Tracking chlorinated contaminants in the subsurface using compound-specific chlorine isotope analysis: A review of principles, current challenges and applications. Chemosphere 244. https://doi.org/10.1016/j.chemosphere.2019.125476