Toxicological and ecotoxicological assessment of water tracers

H. Behrens · U. Beims · H. Dieter · G. Dietze
T. Eikmann · T. Grummt · H. Hanisch · H. Henseling
W. Käß · H. Kerndorff · C. Leibundgut
U. Müller-Wegener · I. Rönnefahrt · B. Scharenberg
R. Schleyer · W. Schloz · F. Tilkes

Abstract Uncertainties regarding possible negative effects on the environment or on human health of authorizing tracing experiments in groundwater and surface waters led to the establishment of a Working Group at the German Federal Environmental Agency (Umweltbundesamt – UBA) for conducting a toxicological and ecotoxicological assessment. A total of 17 water tracers was assessed by the Working Group on the basis of the results of toxicological tests, the available literature, and the group's expert knowledge. In the future, tracers that pose a risk to the environment or to human health should no longer be used. Nevertheless, there are a number of tracers that could be used in hydrogeological and hydrological investigations for water-pollution-control purposes with no adverse environmental impact.

Résumé Les incertitudes concernant les effets négatifs possibles sur l'environnement ou sur la santé de l'homme d'expériences de traçage autorisées dans les nappes et dans les eaux de surface a conduit à créer un groupe de travail de l'Agence Fédérale Allemande de l'Environnement (Umweltbundesamt – UBA) qui a réalisé une évaluation toxicologique et écotoxicologique. Un total de 17 traceurs de l'eau ont été évalués par le groupe de travail à partir des résultats de tests toxicologiques, de la littérature disponible et des connaissances des experts du groupe. Dans le futur, les traceurs qui présentent un risque pour l'environnement et pour la santé de l'homme ne devraient plus être utilisés. Toutefois, il existe un certain nombre de traceurs qui peuvent être utilisés en hydrogéologie et en hydrologie pour le contrôle de la pollution des eaux et qui ne présentent pas de danger pour l'environnement.

Resumen Las incertidumbres respecto a los posibles efectos negativos de los experimentos con trazadores de aguas subterráneas y superficiales en el medio ambiente o en la salud humana llevó al establecimiento de un grupo de trabajo en la Agencia Federal Alemana de Medio Ambiente (Umweltbundesamt – UBA), con el objetivo de efectuar una evaluación toxicológica y ecotoxicológica. El grupo de trabajo estudió un total de 17 trazadores del agua con base en los resultados de los ensayos toxicológicos, la bibliografía disponible y la experiencia de los propios miembros del grupo. En el futuro, no se debería volver a utilizar aquellos trazadores que representen un riesgo para el medio ambiente o la salud humana. Sin embargo, diversos trazadores podrían ser empleados en investigaciones hidrogeológicas e hidrológicas para controlar la contaminación del agua, sin ocasionar un impacto medioambiental desfavorable.

Keywords tracer tests · toxicity · groundwater protection

Introduction

Tracing experiments in groundwater and surface waters are necessary in investigations related to management or protection of water resources, and for scientific investigations. They are used mainly to obtain information on flow, transport, and mixing processes. Such information is needed inter alia for the formulation of sustainable protective measures. Applications include the demarcation of water-protection areas and of catchment areas where drinking water abstraction plants are situated, the tracing of pathways of pollutant dispersal, or the simulation of hazardous incidents. A comprehensive overview of water-tracing technology is provided by Käß (1998).

Tracing experiments also pose a potential risk of water contamination, the avoidance of which must be the subject of careful investigations. In a tracing experiment, a tracer is applied to a body of water intermittently or over a limited time. It spreads in the direction of the water flow and is then detectable at observation points for a limited time.

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H. Behrens · U. Beims · H. Dieter · G. Dietze · T. Eikmann
T. Grummt · H. Hanisch · H. Henseling · W. Käß · H. Kerndorff
C. Leibundgut · U. Müller-Wegener · I. Rönnefahrt
B. Scharenberg · R. Schleyer (✉) · W. Schloz · F. Tilkes
German Federal Environmental Agency Working Group,
Federal Environmental Agency (Langen Branch),
P.O. Box 1468, D-63204 Langen, Germany
e-mail: ruprecht.schleyer@uba.de
Fax: +49-6103-704147

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To be suitable for water tracing, tracers must exhibit specific properties. They should not occur naturally in waters at all, or occur naturally only in low concentrations. They must be readily detectable by analysis, sufficiently persistent, and show an optimal pattern of dispersal with water flow. The following discussion of tracing agents does not deal with their physico-chemical properties and resultant suitability for water tracing. This information is documented by Käß (1998).

A decisive criterion for the suitability of a tracer is its toxicity to humans and its ecotoxicity. It should be remembered in this context that the vast majority of tracing experiments are carried out within catchments where drinking water abstraction plants are situated. Groundwater dosed with tracers can reach surface waters via springs and exfiltration and, conversely, such water can end up in the drinking water supply system by way of bank-side filtration and direct abstraction of surface water. Thus, a general principle is that in field studies only tracers that have been tested for their toxicity to humans and the environment, and found acceptable in these respects, should be used.

The data available on many tracers are incomplete or not readily comparable (e.g. Smart and Laidlaw 1977; Smart 1984; overview by Käß 1998). They do not suffice for a use-oriented toxicological assessment. Therefore, the authorities responsible for authorising tracing studies in some instances have denied authorization or only granted it subject to considerable restrictions. Conditions imposed may include, for example, adherence to very low concentrations of the tracing agent, which, for hydrogeological, hydrological, or analytical reasons, is often not feasible in the framework of the planning and performance of a tracing study.

These uncertainties led to the establishment of a Working Group at the German Federal Environmental Agency, which was composed of experts in the fields of hydrogeology, hydrology, human toxicity, ecotoxicity, pedology, and chemistry and aimed at assessing the most frequently used tracing agents with respect to their toxicity to humans and their ecotoxicity. The assessment of possible evolution of degradation and transformation products was not the aim of the Working Group; this question requires further investigation.

Following is a list of the members of the Working Group (in alphabetical order):

- Mr. Behrens (University of Munich).
- Prof. Dr. Beims, representing the German Association of Gas and Water Experts (DVGW).
- Dr. Dieter, Dr. Grummt, Dr. Kerndorff, Prof. Dr. Müller-Wegener, Dr. Rönnefahrt, Mr. Scharenberg, and Dr. Schleyer, representing the German Federal Environmental Agency (Umweltbundesamt – UBA).
- Dr. Dietze and Dr. Schloz, representing the Geological Survey of the federal state Baden-Württemberg.
- Prof. Dr. Eikmann and Dr. Tilkes (University of Gießen)
- Mr. Hanisch, representing the German Federal Institute of Hydrology (BfG)
- Prof. Dr. Henseling, representing the Working Group of the Chief Medical Officers of the German Federal States (AGLMB)
- Prof. Dr. Käß, representing the German Association for Water Resources and Land Improvement (DVWK)
- Prof. Dr. Leibundgut (University of Freiburg).

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Test Methods Used

The results of tests to evaluate the geno- and ecotoxicity of the tracers, shown in Table 1, were performed by the Federal Environmental Agency. Commercial tracers were used for this purpose; many of these are not pure substances but contain additives, in some cases in substantial amounts.

For substances with low-level or avoidable human exposure, a premise that is assumed to hold true in the case of tracing agents, a combination of two to three in-vitro test methods with relevant genetic end points is considered sufficient to cover genotoxic effects (Kramers et al. 1991; Kirkland 1993). Two test methods that have been sufficiently validated and established for testing of chemicals were used: the salmonella/microsome test (gene mutation) and the analysis of chromosome aberration using a mammalian cell culture (chromosome aberration).

Ecotoxicological assessments were based on the determination of acute toxicity to daphniae according to DIN 38412, Part 11 (Deutsches Institut für Normung e.V. 1982), and acute toxicity to fish according to DIN 38412, Part 31 (Deutsches Institut für Normung e.V. 1989). For the latter, zebra fish were used as a modification of the guideline. Due to interferences caused by the fluorescence of most tracers, the algae test required as a base-set test for the ecotoxicological assessment of chemicals under the German Chemicals Act (1994) provided no conclusive results and therefore could not be used for ecotoxicological assessments.

The selection of test concentrations was geared to the study-specific concentration patterns as used in tracing practice. The highest concentrations tested were in milligrams per liter.

Test Results and Assessment

Fluorescent Dyes

The results of the toxicological tests performed on behalf of the Working Group are compiled in Table 1. Fluorescent dyes that showed no effect upon either the genotoxicity or the ecotoxicity tests were classified by the Working Group as safe for use in water tracing. These are:

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Table 1 Results of the toxicological tests performed on tracers on behalf of the Working Group. *Minus signs* under Genotoxicity indicate a negative test result; under Ecotoxicity a lethal concentration (LC_0) with no affected individuals (fish) and EC_0 (*daphniae*) >10 mg/L respectively; *plus signs* under Genotoxicity indicate a positive test result; under Ecotoxicity LC_0 (fish) and EC_0 (*daphniae*) <10 mg/L, respectively; *n.t.* no tests performed; test results reported in the literature are negative

Tracer	Genotoxicity		Ecotoxicity	
	Salmonella/ microsome test	Cytogenetic analysis	Fish test	Daphniae test
Uranine	–	–	–	n.t.
Eosin yellow	n.t.	n.t.	–	n.t.
Sulforhodamine B	–	–	–	+
Amidorhodamine G[a]	-	–	–	–
Rhodamine WT	+	+	–	–
Rhodamine B	–	+	–	–
Rhodamine 6G	+	+	+	–
Sodium naphthionate	–	–	–	–
Pyranine	–	–	–	–
Tinopal CBS-X	–	–	–	–
Tinopal ABP liquid	–	–	–	–
Polystyrene microspheres	–	–	n.t.	n.t.
Spores of club moss	–	–	n.t.	n.t.

[a] In addition, amidorhodamine G was tested by the Federal Institute of Hydrology for toxicity to algae according to DIN 38412, Part 33 (Deutsches Institut für Normung e.V. 1991), using color-correction cuvettes in modification of the guideline; the test result was negative

uranine, eosin yellow, amidorhodamine G, sodium naphthionate, pyranine, tinopal CBS-X, and tinopal ABP liquid. Fluorescent dyes for which effects were observed in at least one of the tests are discussed as follows.

Sulforhodamine B
Sulforhodamine is safe in terms of human toxicity, but ecotoxicologically unsafe. The EC_0 (EC = effect concentration; index = percent of affected individuals) for daphniae at an exposure period of 48 h is 0.16 mg/L [EC_{50} (48 h)=0.7 mg/L]. Therefore, its use in water tracing is justifiable only following specific evaluation of each individual experiment.

Rhodamine WT
As it has genotoxic properties, the use of rhodamine WT for water tracing cannot be recommended.

Rhodamine B and rhodamine 6G
Because of genotoxic and ecotoxic properties, rhodamine B and rhodamine 6G cannot be recommended for use for water tracing. Mutagenic and carcinogenic effects had already been found in earlier investigations (Mull et al. 1988).

Salts
The cations lithium and strontium, which can be used for water tracing, are trace elements present in every natural body of water. They are normally not determined in routine water analyses. The concentration of strontium in groundwater and surface water is, on average, about 100-fold that of lithium.

Lithium salts
It is known from human medicine that lithium (Li) has pharmacological effects which are used, in particular, for the treatment of psychological disorders (Schrauzer and Klippel 1991). In addition, there are indications that lithium chloride is ecotoxicologically unsafe, which, however, could not be clarified conclusively by the Working Group. Therefore, in tracing experiments, lithium concentrations in resurfaced groundwater and in surface water should be kept as low as possible.

Concentrations of Li in drinking water, regarded as safe by the Working Group, are 5 mg/L as a short-term maximum persisting for not more than 1 week and 0.5 mg/L as a long-term maximum. Experience has shown that in tracing experiments with lithium salts this requirement can be satisfied without any difficulty.

Strontium salts
Salty groundwater, so-called gypsum water, naturally contains elevated concentrations of strontium (Sr). In addition, drinking water from the northern part of the federal state of Hessen (central Germany), from aquifers in the Lower Muschelkalk (Middle Triassic) or in the Keuper (Upper Triassic) is known to contain strontium in concentrations of up to 15 mg/L. The Working Group has no objections to tracing experiments with strontium salts, as long as a concentration of 15 mg/L is not significantly exceeded in drinking water.

Bromides
Bromine is also a trace element invariably present in natural waters. However, tracing experiments with bromine salts can result in elevated concentrations of the genotoxic bromate and the formation of potentially carcinogenic brominated organic compounds in drinking-water supply plants that use chlorination and ozonization treatment. In such cases, the Working Group advises against the use of bromides as tracers.

Activatable Isotopes
Elements of this type which can be readily activated and normally occur in natural waters in concentrations well below the detection limit for conventional analytical procedures are used as tracers. In water tracing, rare-earth metals as well as the element indium (In) are used. They are usually used in water tracing in quantities of less than ten to several hundreds of grams as highly stable

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anionic complexes. Following activation, usually in a research reactor using neutrons, they are detected in water samples by measuring the short-lived radioactivity generated from them.

The element concentrations at resurfacing sites can be expected to be well below the effective concentrations known from toxicology. However, the complexing agents ethylenediaminetetraacetic acid (EDTA) and diethylenetriaminepentaacetic acid (DTPA) must be taken into account. A non-statutory guide value of the Drinking Water Commission of the UBA for the complexing agent EDTA is 10 µg/L. However, this is based not on a toxicological but exclusively on a hygienic and ecological rationale. The Working Group regards tracing experiments with these tracers as safe, provided it is ensured that the concentration of the complexing agents in drinking water does not exceed 10 µg/L.

Drift Particles

These tracers are usually used in karst terranes. Their advantage is that they remain invisible at resurfacing sites even when present in fairly high concentrations. The results of drift experiments are strongly dependent upon the filtration properties of the rock through which the water flows.

Fluorescent polystyrene microspheres

So far, fluorescent polystyrene microspheres have been tested in different karst waters with good success. Flow paths of pathogenic germs can be traced with spheres 1 µm in diameter. Concentrations of 100 spheres/L of investigated water are rarely exceeded in practice, and then only for a short time. The fluorescent dye used to identify the spheres is inseparably bound to the polystyrene. Such spheres are also used in human medicine for blood-circulation investigations. The aqueous extracts proved to be non-genotoxic (Table 1). The Working Group recommends that experiments with fluorescent microspheres be authorized where shown to be warranted and carefully planned.

Spores of Lycopodium

Spores of club moss (*Lycopodium clavatum*) are usually used in karst terranes where springs are not readily accessible. Unlike dissolved tracers they can be accumulated over prolonged observation periods using plankton nets. For easier detection in samples they can be colored with fluorescent dyes, one of which, acridine orange, has proved to be the most adhesive. Shake tests have shown that no detectable amounts of acridine orange are released into water from spores dyed with it. Tests with these aqueous phases showed no genotoxic effects (Table 1). Therefore, there are no objections to tracing experiments with club-moss spores dyed with acridine orange.

Conclusions

In some cases, tracing experiments involve an intentional introduction of xenobiotic substances into waters and are thus a potential source of water contamination. Therefore, each individual experiment requires detailed justification and planning in terms of its necessity, type, quantity of tracer to be used, and the soundness of the results. The benefit expected to be derived from the results of the experiment must be carefully weighed against possible impairments to water bodies. As a general rule, water tracing must be minimized in terms of the number of experiments, number of tracers used, amount of tracer applied, expected concentration in resurfaced water, and the exposure period.

Particular care must be exercised when tracing experiments are performed within the catchment areas of drinking-water abstraction plants. The visibility threshold of coloring tracing agents must not be exceeded in drinking water (German Drinking Water Ordinance 1990, Annex 4, Coloration). The tracers assessed here as

Table 2 Overview of toxicological assessment and basis for assessment of the 17 tracers considered by the Working Group. *T* Toxicological tests; *L* literature search; *W* Working Group's expert judgement

Tracer	Toxicological assessment	Assessment basis
Uranine	Safe	T, L
Eosin yellow	Safe	L, W
Sulforhodamine B	Ecotoxicologically unsafe	T
Amidorhodamine G	Safe	T
Rhodamine WT	Not recommended	T
Rhodamine B	Not recommended	T, L
Rhodamine 6G	Not recommended	T, L
Sodium naphthionate	Safe	T
Pyranine	Safe	T
Tinopal CBS-X	Safe	T
Tinopal ABP liquid	Safe	T
Lithium salts	Safe with restrictions	L, W
Strontium salts	Safe with restrictions	L, W
Bromides	Safe with restrictions	L, W
Activatable isotopes	Safe with restrictions	L, W
Fluorescent polystyrene microspheres	Safe	T, W
Spores of club moss dyed with acridine orange	Safe	T, W

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safe from the standpoint of human toxicology and ecotoxicology or as safe with restrictions shown in Table 2 provide a sufficient number of tracers to tackle almost every tracing task. In the future, new tracing agents should generally be required to undergo toxicological and ecotoxicological assessment prior to their application to water sources.

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