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Water quality — Guidance on statistical interpretation of ecotoxicity data

Qualité de l'eau — Lignes directrices relatives à l'interprétation statistique de données écotoxicologiques

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Contents

Page

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

In other circumstances, particularly when there is an urgent market requirement for such documents, a technical committee may decide to publish other types of normative document:

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An ISO/PAS or ISO/TS is reviewed after three years in order to decide whether it will be confirmed for a further three years, revised to become an International Standard, or withdrawn. If the ISO/PAS or ISO/TS is confirmed, it is reviewed again after a further three years, at which time it must either be transformed into an International Standard or be withdrawn.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO/TS 20281 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

Introduction

Ecotoxicity tests are biological experiments performed to examine if either a potentially toxic compound, or an environmental sample (e.g. effluent, sediment or soil sample) causes a biologically important response in test organisms. If so, the goal is to determine the concentration that produces a given level of effects or produces an effect that cannot be distinguished from background variation.

In a test, organisms are exposed to different concentrations or doses of a test substance or a test substrate (e.g. waste water, sludge, or a contaminated soil or sediment), sometimes diluted in a test medium. Typically, at least one group of test organisms (the control group) is not exposed to the test substance or substrate, but is otherwise treated in the same way as the exposed organisms.

The endpoint(s) observed or measured in the different batches may be the number of surviving organisms, size or growth of organisms, number of eggs or offspring produced or any relevant biochemical or physiological variable that can be reliably quantified. Observations are made after one or several predefined exposure times. The endpoint's relationship with the concentration of the tested chemical or substrate is examined. The way statistics are applied may have a considerable impact on the results and conclusions from ecotoxicity tests, and consequently on the associated policy decisions. Various documents (Williams 1971, Piegorsch and Bailer 1997, Tukey *et al*. 1985, Pack 1993, Chapman *et al*. 1995, Hoekstra 1993, Kooijman and Bedaux 1996, Laskowkj 1995, Chapman 1996, OECD 1998, ASTM 2000) exist on the use of available statistical methods, the limitations of these methods and how to cope with specific problematic data. Discussions of statistical principles and commonly used techniques are found in general references such as Armitage and Berry (1987) [basic information on hypothesis testing and regression, transformations], Finney (1978) [analysis of quantal data, especially probit models], Hochberg and Tamhane (1987) [thorough treatment of multiple comparison methods], Newman (1994) [information related to biology-based models, EC_x], and Sparks (2000) [a collection of articles covering field and laboratory experiments, multivariate techniques, risk assessment, and environmental monitoring].

When problematic data are encountered or critical decisions depend upon inferences from ecotoxicity tests, consultation with a qualified statistician is useful. (Statisticians should be consulted before beginning the experiment to ensure proper design, sample size, limitations, and to be sure that the study is actually able to answer the research question that the experimenter poses. Once bad data have been collected, there is little a statistician can do to rectify the problem.)

Clause 8 contains a table listing all the existing ISO and OECD ecotoxicity standards/guidelines that could be analysed using this guidance document. For each standard/guideline, reference is made to the adapted clauses of this Technical Specification.

Clause 4 details the different statistical approaches that can be used for the analysis of ecotoxicity data, depending on the aim. In particular, it gives the assumptions made when using hypothesis-testing methods, concentration-response modelling methods or biology-based methods and their limitations. It also gives some indication on experimental design issues. Some general principles and advice are also given for the process of data analysis.

Clause 5 deals with hypothesis testing, Clause 6 with dose-response modelling and Clause 7 with biology-based methods.

There was an ISO resolution (ISO TC 147/SC 5/WG 10 Antalya 3), as well as an OECD workshop recommendation (OECD 1998), that the NOEC should be phased out from International Standards.

However, the NOEC is still required in many regulatory standards from many countries, and in some cases, where a detailed determination of an EC_x is not relevant and the alteration of the study design is too costly to fulfil the requirements for regression models. Therefore guidance is provided on the statistical methods for the determination of the NOEC.

In the annexes, examples of analyses with the three main methods (hypothesis testing for NOEC estimation, dose-response modelling and biology-based modelling) of four different data sets are given. They concern:

— acute toxicity on *Daphnia magna*;

— inhibition of algae growth;

— reproduction of *Daphnia magna*; and

— fish growth.