

TECHNICAL REPORT

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Toxicity testing of fire effluents —

Part 2:

Guidelines for biological assays to determine the acute inhalation toxicity of fire effluents (basic principles, criteria and methodology)

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Essais de toxicité des effluents du feu —

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Partie 2: Directives pour les essais biologiques permettant de déterminer la toxicité aiguë par inhalation des effluents du feu (principes de base, critères et méthodologie)



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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.

The main task of technical committees is to prepare International Standards, but in exceptional circumstances a technical committee may propose the publication of a Technical Report of one of the following types:

- type 1, when the required support cannot be obtained for the publication of an International Standard, despite repeated efforts;
- type 2, when the subject is still under technical development or where for any other reason there is the future but not immediate possibility of an agreement on an International Standard;
- type 3, when a technical committee has collected data of a different kind from that which is normally published as an International Standard ("state of the art", for example).

Technical Reports of types 1 and 2 are subject to review within three years of publication, to decide whether they can be transformed into International Standards. Technical Reports of type 3 do not necessarily have to be reviewed until the data they provide are considered to be no longer valid or useful.

ISO/TR 9122-2, which is a Technical Report of type 2, was prepared by Technical Committee ISO/TC 92, *Fire tests on building materials, components and structures*.

ISO 9122 consists of the following parts, under the general title *Toxicity testing of fire effluents*:

- *Part 1: General*
[Technical Report]
- *Part 2: Guidelines for biological assays to determine the acute inhalation toxicity of fire effluents (basic principles, criteria and methodology)*
[Technical Report]
- *Part 3: Methods for the analysis of gases and vapours*

Annex A of this part of ISO 9122 is for information only.

Introduction

Several small-scale test methods for assessing the inhalation toxicity of the fire effluents of materials and simple composites have been described in the literature. They have been used mostly for research and development purposes.

Such test methods are usually split into three parts: a fire model (generation of fire effluent), analytical methods, and an animal model (biological assay procedure). These test methods differ substantially, especially in the use of various fire models.

ISO/TR 9122-2 considers only the biological assay procedures (animal model). The approach used in this document has been to recommend minimum standards of scientific practice. This principle has been successfully applied in the toxicity assessment of drugs, pesticides and chemicals and, as a consequence, international guidelines harmonizing scientific contributions in toxicity testing have been published.

The guidelines in this Technical Report for the determination of the acute toxicity of fire effluents have been developed from the collective experience of the participating experts and from their consideration of published results as shown in the bibliography (see annex A).

Basic principles of inhalation toxicology (as outlined, for instance, in international guidelines for toxicity testing of chemicals, pesticides or drugs) also apply to the determination of the acute inhalation toxicity of fire effluents. Additionally, criteria have been determined for acceptable biological assays which consider specific effects in combustion toxicology. Some criteria have been defined from the toxicologist's point of view concerning acceptable fire models (see clause 2) and suitable analytical methods. Recommendations for an appropriate selection of suitable methods have been formed by critically reviewing biological assay procedures against these basic principles and special criteria.

The following basic principles and criteria have been selected and are discussed:

- the nature of toxic effects (narcosis, irritancy, etc.);
- the relevance of animal data to humans;
- suitable endpoints of biological assays (lethality and incapacitation);
- characterization of toxic effects (qualitatively and quantitatively);
- reliability, validity, repeatability, reproducibility and sensitivity;
- characterization of doses;
- exposure time period (5 min and 30 min);

- exposure systems;
- modes of exposure;
- fire models;
- observations and examinations;
- *post mortem* examination;
- data evaluation and reporting;
- good laboratory practice;
- personnel.

Test concepts are recommended and the following conclusions are drawn:

- suitable biological assays are available for the determination of narcotic effects which meet the basic principles and criteria;
- biological assays are available for the determination of sensory irritant effects which meet the basic principles and criteria. The correlation of the effects found in animals with humans is uncertain;
- suitable biological assays are available for the determination of pulmonary irritant effects, which meet the basic principles and criteria;
- suitable biological assays such as the OECD (Organisation for European Community Development) Guidelines [2] are available for the determination of toxic effects other than narcotic or irritant ones, which meet the basic principles and criteria;
- analytical pretests are recommended before tests with animals are performed in order to minimize the use of animals.

Toxicity testing of fire effluents —

Part 2:

Guidelines for biological assays to determine the acute inhalation toxicity of fire effluents (basic principles, criteria and methodology)

1 Scope

The main objective of ISO Technical Report 9122-2 is to provide researchers with basic background information on methods suitable to define the acute inhalation toxicity of fire effluents, as generated by fire models (see clause 2).

In producing ISO Technical Report 9122-2, comprehensive and critical reviews have been made of the current state-of-the-art of biological assay methods in combustion toxicology. It is, therefore, hoped that researchers will be encouraged to use common approaches in research, so that data and test results can be more widely used for assessments of comparative toxicity, and also to minimize the overall use of biological assays.

While it has been felt essential to specify minimum standards of scientific practice, the selection of suitable and recommended experimental methods is left to the judgement and responsibility of the scientific experts performing these tests.

The scope of this Technical Report includes

- basic principles of inhalation toxicology applicable for biological assay of fire effluents;
- criteria for acceptable biological assays;
- some criteria on fire models and analytical methods, from the toxicologist's point of view.

This Technical Report does not take into account chronic and long-term effects of fire effluents and their adverse effects, such as heat or oxygen depletion, arising from fire.

This Technical Report is mainly intended to be useful in research and development laboratories. It should be emphasized that the use of toxicity test results alone to classify materials with respect to their fire safety use is inadequate. The integration of toxicity data into a toxic hazard assessment is essential but is presently not well defined and should be the next major goal for combustion toxicology.

2 Definitions

For the purposes of this Technical Report, the following definitions apply.

2.1 acute toxicity: The effects following a single exposure to, or dose of, a toxicant. The effects may be seen immediately, or after a delay of hours or days.

2.2 biological assay (or bioassay): Originally a term reserved for the use of a biological system to detect and/or measure the amount of a biologically active material. In the fire context it refers to the use of animal exposures, rather than chemical analyses, to determine the toxicity of a fire effluent.

2.3 chronic toxicity: Toxicity resulting from multiple doses or exposure to a toxicant over an extended period of time.

2.4 concentration: The amount of a contaminant in the atmosphere per unit of volume of the atmosphere, usually quoted as mass/volume (milligrams per millilitre or milligrams per cubic metre) or volume/volume (parts per million or per cent).

2.5 delayed toxicity: A toxic effect which is not manifested until a period of several hours, days or, in some cases, weeks after exposure to the toxicants.

2.6 dose: The amount of a toxicant received by an animal. In combustion inhalation toxicology, the dose can be determined in terms of the Exposure-Dose.

2.7 EC₅₀: Effective concentration 50 %. A concentration statistically calculated to cause an effect (e.g., incapacitation) in 50 % of the exposed animals.

2.8 ECT₅₀: The mathematical product of time of exposure and concentration statistically calculated to cause an effect in 50 % of the animals.

2.9 exposure-dose: The amount of toxicants to which a subject is exposed for a specified time period. For individual gaseous toxicants, the Exposure-Dose is the integrated area under a concentration vs. time curve and is expressed in parts per million minutes. In combustion toxicology of fire effluents, the Exposure-Dose is the integrated area under a mass loss per unit volume vs. time curve and is expressed in milligrams minutes per litre. It is often estimated by multiplying the concentration (expressed as the nominal furnace load/chamber volume or nominal mass loss/chamber volume) by the time of exposure.

2.10 fire effluent: The total gaseous, particulate or aerosol effluent from combustion or pyrolysis.

2.11 fire model: A means for the decomposition and/or combustion of test specimens under defined conditions to represent a known stage or stages of fire in order to generate fire effluents for toxicity assessments. (This term is also used by the fire science community to mean the mathematical simulation of fire characteristics.)

2.12 incapacitation: An inability to perform a task (related to escape from a fire) caused by exposure to toxicants.

2.13 irritation (pulmonary): The action of irritants on the lower respiratory tract which may result in breathing discomfort (dyspnoea), increase in respiratory rate and, in severe cases, to pneumonitis or pulmonary oedema.

2.14 irritation (sensory): A response evoked in the eyes and upper respiratory tract by a toxicant and causing a painful sensation. This may be a direct stimulus of specialized receptors or secondary to tissue damage caused by the toxicants.

2.15 LC₅₀: Lethal concentration 50 %. The concentration statistically calculated to cause the death of

half the animals exposed to a toxicant for a specified time. It may be expressed in parts per million (ppm) (by volume), or milligrams per litre (mg/l). In combustion toxicology, two values are often used: a) the LC₅₀, expressed as milligrams per litre which is the starting mass of material in the study divided by the volume of available air (nominal furnace load concentration), and b) the LC₅₀ expressed as milligrams per litre which is the mass of material actually consumed (i.e., the difference between the starting mass and the finishing mass) divided by the volume of available air (nominal mass loss concentration). Care must be taken to distinguish between the two. As explained under Exposure-Dose, the time of exposure is very important in inhalation toxicology, and the length of exposure should always be quoted for an LC₅₀.

2.16 LCT₅₀: The Exposure-Dose statistically calculated to cause the death of 50 % of the animals. This value is useful for comparing results obtained in experimental regimes employing different exposure times. The duration time of exposure must always be quoted.

2.17 LT₅₀: The time exposure statistically calculated to cause the death of 50 % of the animals for a fixed concentration of toxicant.

2.18 narcosis: Literally "sleep inducing", but used in combustion toxicology to describe central nervous system depression causing reduced awareness and reduced ability to escape. At higher concentrations of toxicants, unconsciousness and finally death will occur.

2.19 pneumonitis: Inflammation of the lower respiratory tract.

2.20 pulmonary oedema: Extravasation of blood plasma in the alveolar regions of the lung caused by vascular damage, inflammation or inadequate venous drainage. The build-up of fluid impairs the absorption of oxygen into the blood.

2.21 respiratory tract: The nose, pharynx, larynx, trachea and large bronchi are termed the upper respiratory tract and the bronchioli, alveolar ducts and alveoli are termed the lower respiratory tract.

2.22 specific toxicity: A particular adverse effect caused by a toxicant (e.g., narcosis, irritancy).

2.23 toxic potency: A measure of the amount of toxicant required to elicit a specific toxic effect — the smaller the amount required, the greater the potency.

2.24 toxicant: A chemical capable of exerting an adverse effect or effects on an organism. The toxicant can be characterized by two properties: the specific toxicity — the nature of the adverse effect,

and the toxic potency — the dose required to cause the effect.

2.25 toxicity: The nature (specific effect) and extent (potency) of adverse effects of a substance upon a living organism.

3 Principles

3.1 Nature of toxic effects

The aim of most toxicity evaluations is to provide data in order to predict the consequences of exposure in humans. Suitable available data concerning the effect of similar substances on humans must be considered in determining the relevance of animal studies. Data exist, concerning the effect of fire effluent atmospheres, which have been derived from studies on many fire victims, especially *post-mortem* examinations. Victims are generally found with high carboxyhaemoglobin levels, indicating exposure to carbon monoxide, and in some cases hydrogen cyanide exposure has been implicated. Carbon monoxide and hydrogen cyanide are both known to cause progressive central nervous system depression leading to unconsciousness and death. This type of toxic effect has been termed "narcosis" and is considered to be very important in the response of humans to fire effluents.

There are many reports of fire effluents being described as "irritant", causing coughing, choking and an inability to see. Chemical pneumonitis has also been reported in both fire survivors and fatalities. Irritancy, both sensory and pulmonary, is considered to be a major factor in the response of humans to fire effluents. There have been few, if any, fire casualty reports due to other significant toxicological effects apart from narcosis and irritancy.

3.2 Relevance of animal data to humans

The effects seen in experimental animals have been very similar to those seen in humans. Death has been attributed to the presence of "narcotic" gases such as carbon monoxide and hydrogen cyanide. Irritants have been shown to be present, detected by clinical observations of salivation, nasal discharge, lachrymation and measurements of respiratory rate. Pulmonary damage has also been confirmed by histopathological examination of the lungs of animals exposed to high concentrations of corrosive irritants.

In general, direct-acting chemicals appear to have the same spectrum of activity across the species, including humans. A comprehensive review [5] compared the action in humans and laboratory animals for an extensive list of chemicals present in fire effluents and, in many cases, agreement was found

between the acute effects in humans and the effects in laboratory animals.

There are, however, some physiological differences between small rodents and man which are especially important in combustion toxicology. For instance, the respiratory minute volume to body weight ratio of small rodents is generally greater than in humans. This phenomenon has been used to human advantage in the past where small rodents or canaries have been used to detect the presence of narcotic gases in mines and chemical vessels. The rodent is adversely affected before a human because, at rest, a human breathes 160 ml/(min·kg) whereas a rat breathes 900 ml/(min·kg) — a ratio of approximately 1:6. However, under conditions of activity, such as may be encountered in a fire, a human's respiration rate can increase to 500 ml/(min·kg) or even higher, giving a ratio of approximately 1:2. Thus it could be considered that the rat provides a reasonable model of "active" human respiratory uptake. Any discrepancy resulting from rats being more sensitive than humans can be tolerated as this provides a "safety factor" when extrapolating the results from animals to humans. It would also be unrealistic to expect the precision of the response in laboratory animals, to a complex mixture of compounds present in fire effluents, to be such that a factor of 2 difference between rat and human would significantly affect the extrapolation of the data.

A second physiological difference of importance is that small laboratory rodents are obligate nose breathers, whereas a human can choose to breathe through either the mouth or the nose. In fact, under conditions of high workload, stress, or in the presence of irritants, man becomes almost totally a mouth breather. This difference is of little or no importance with regard to the effects of insoluble gases, but the rodent nose acts as a "scrubber" to remove water soluble gases, such as SO₂ or HCl and particulates. This may reduce the effect of these materials upon the lower respiratory tract. However, fine particles (less than 5 µm) and high concentrations of soluble gases will still have a significant impact upon the lungs in rodents as well as humans.

In spite of these differences, the qualitative relationship between the effects of fire effluents on humans and laboratory animals is excellent and a reasonably good quantitative correlation has been observed for fire effluents studies to date.

3.3 "Classical" inhalation toxicology vs. combustion toxicology

Most acute inhalation toxicology studies are carried out as part of the toxicological characterization of a chemical to enable decisions to be made about its use, transport and safe exposure levels. The aim of these considerations is the protection of those mak-

ing, using or buying the chemical. Consequently, rigorous testing régimes are used to provide "worst-case" assessments and 1-h or 4-h exposures are often used. The level of adverse effects which will be tolerated is very low, so that mere survival at a concentration is not considered adequate if there are other, non-fatal consequences. The range of toxic effects which are likely to occur is very broad and the protocols employed are designed to reflect this. In contrast, combustion toxicology aims to model an "emergency" situation. Consequently, the exposure times used are generally much reduced, 5 min to 30 min being typical. Survival is the single most important factor and consequently much greater emphasis is placed on incapacitation, severe toxicity and death, rather than changes in body weight, for instance, which would be significant in a conventional study. As knowledge in combustion toxicology has increased, the range of toxic effects considered to be important has narrowed to narcosis and irritation. Specific consideration must be given to these.

3.4 Determination of qualitative aspects of toxicity (specific toxicity)

Toxicology involves the determination of the biological responses caused by toxicants in both qualitative and quantitative terms. The OECD acute inhalation protocols [2] have been devised to provide a broad screen for the determination of the qualitative aspects of toxicology, for instance, whether the toxicant causes death by narcosis, or liver, kidney or pulmonary damage. This can be considered to be the determination of the toxicant's specific toxicity (see clause 2).

3.5 Determination of quantitative aspects of toxicity (toxic potency)

The quantitative aspects of a toxicant are addressed by varying the dose of toxicant and relating it to the toxicity seen. At each dose level the effects are recorded either as quantal or continuous variables. The two values most commonly use are the ED₅₀ (effective dose to cause 50 % response or a response in 50 % of the animals) and the "No Effect Level" (the highest dose which does not cause a particular effect). In inhalation toxicology, including combustion toxicology, the EC₅₀ type of value is most often calculated, for instance the LC₅₀ (*t*) (concentration to which the animals have been exposed for a time *t*, and which is calculated to kill 50 % of the animals) or the incapacitation EC₅₀ (*t*) (the concentration to which the animals have been exposed for a time *t* which is calculated to cause incapacitation in 50 % of the animals). These values are calculated from experiments where responses of less than 50 % have resulted and from experiments where more than 50 % responses have occurred.

Both the LC₅₀ and the EC₅₀ are then calculated statistically.

In addition to the commonly quoted LC₅₀ and EC₅₀ values, two other values are determined: the slope of the concentration-response curve and the confidence limits for the LC₅₀ and EC₅₀. The slope of the concentration-response curve is important; for instance, two toxicants with the same LC₅₀ may have different effects at a fraction of the LC₅₀. A compound with a steep concentration-response curve would probably have no effect at 0,2 LC₅₀, whereas one with a shallow curve may cause death in 20 % of the animals at 0,2 LC₅₀. In most combustion toxicity experiments the concentration-response curves have been steep. The confidence limits for the LC₅₀ describe the certainty with which a given figure lies within a range. The inherent variability of LC₅₀ determinations is such that differences of less than a factor of 2 to 3 are often not statistically significant; in addition, such a factor would not be toxicologically significant.

3.6 Relative toxicity and its significance

The quantitative indices of toxicity are often used to compare the toxicities of different materials to assess their relative toxicity. This can be misleading unless it is certain that similar values are being compared (see 3.5 for some of the problems associated with the expression of "dose" and LC₅₀ values in combustion toxicology). Even when care has been taken to express the values in an appropriate way, practical differences in relative toxicity are usually not indicated unless LC₅₀s differ by greater than one order of magnitude. While reported LC₅₀s for individual gases found in fire effluents are distributed over several orders of magnitude, LC₅₀s attributed to the fire effluents derived from most materials, expressed as either mass loss or furnace load per unit of exposure volume (milligrams per litre), tend to fall into a much narrower range (1 to 1,5 orders of magnitude). This narrow LC₅₀ range, combined with the inherent variability of LC₅₀ values derived for fire effluents from materials, exerts an obvious limitation on the numbers of categories into which the LC₅₀ values can be classified for significant and practical discrimination of relative toxicity.

3.7 Concentration/time/response relationships

The magnitude or severity of most biological effects increases with increasing doses of the causative agent, usually the increase in effects being proportional to the logarithm of the dose. In inhalation toxicology, the "dose" is a function of many factors, two of which are: the concentration of the toxicant in the atmosphere and the duration of the exposure [10]. Multiplying the atmosphere concentration by the exposure time enables a rough estimate to be made of the "dose" inhaled (but not necessarily re-

tained) by an animal, assuming constant ventilation. Most inhalation toxicology studies use a fixed time of exposure and the dose-response relationship of the compound is investigated by varying the atmospheric concentrations. The magnitude of the response (which may be quantal or continuous) is plotted graphically against the logarithm of the atmospheric concentration. The slope of the relationship can be determined, as can such parameters as the EC_{50} , the atmosphere concentration calculated statistically to cause an effect in half the animals for a quantal response or to cause 50 % of a given continuous effect. Alternatively, the atmosphere concentration can be fixed and the time of exposure varied. In this case, ET_{50} values, the exposure time to cause 50 % responses can be derived. Families of curves relating effect to atmosphere concentration and time of exposure can be generated.

At longer time periods the variation in response is most sensitive to changes in atmospheric concentration, while for short time periods the response is relatively insensitive to changes in atmospheric concentration. Different toxicants will have different time-concentration relationships, which should be understood to characterize the response adequately. This is especially important in combustion toxicology as many fire safety systems use time available for escape as a design criterion.

3.8 Test concepts

The role of testing should basically be to confirm that the toxicity of a fire effluent atmosphere can be adequately described by a consideration of the known constituents and that no other toxicants are present in toxicologically significant amounts, which would cause an unexpectedly high toxic potency.

As a result of considerable research on the common fire effluent toxicants, concentration-time-response mathematical relationships are now becoming quite well understood. Effects of the narcosis-producing toxicants, CO and HCN, on rats, along with reasonable extrapolation to humans via nonhuman primate studies, may be predicted from analytical determination of the time course of toxicant evolution. Current research is aimed at answering questions of interaction. In addition, concentration-time-response relationships for irritant combustion products (HCl, in particular) are now being worked out to enable prediction of irritant effects from analytical data.

It thus appears likely that assessment of both incapacitation and lethal effects of fire effluents containing the common toxicants will not require the use of live animal models for predicting their toxicological effects. The role of a toxicity test using animals would be primarily to validate effects predicted from mathematical models. Conceptually, a sequence for the testing of a material would be as follows:

- a) consideration of the chemical composition of the material to suggest which fire effluent components should be analysed;
- b) thermal decomposition and/or combustion using the method of choice, without exposure of animals, but with analysis for the selected toxicants;
- c) assessment of analytical data to select that set of exposure conditions predicted to cause incapacitation and/or death;
- d) thermal decomposition and/or combustion to produce the predicted effective exposure conditions, with both analysis and exposure of experimental animals using the same method as chosen in b) above. If the animals respond in the predicted manner, the computed data can be considered to be validated. If the animals respond differently from the prediction, either in terms of specific toxicity or the effects occurring at an unexpected time, or with unexpected severity, further work would be required to identify the reason.

This philosophy of testing for the toxicity of fire effluents renders the concepts of "pass" or "fail" as irrelevant. The test concept demonstrates whether or not data derived from a study of the material in relevant fire scenarios can be used to predict threat to life from toxic hazard, in terms of toxicity expressed in the amount of material consumed and/or the toxic gases produced. Used in this way, data from toxicity tests can make a significant contribution by enabling the risk to life in fires to be understood.

The use of animal experiments in this selective way would have other significant consequences. It would reduce the number of experimental animals used and would reserve their use to where it is essential. Additionally, animal experimentation is very expensive, another reason for reserving their use to those occasions where it is absolutely necessary. The economic burden carried by manufacturers, and ultimately by society, during the development of new materials can then be kept to a minimum, while being consistent with safety.

4 Criteria

In order to judge the suitability of a biological assay or test system it must fulfil certain criteria, which are discussed below.

4.1 General criteria

In general, the biological assay must be capable of defining both the qualitative nature of the toxicity caused by fire effluents and the quantitative aspects of the toxicity of fire effluents. The method must use