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)

Essais de toxicité des effluents du feu —

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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Contents

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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 Technical Reports of type 3 do not necessarily have to be reviewed until the data they provide are considered to be no longer valid or useful.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 EC_{50}: Effective concentration 50 %. A concentration statistically calculated to cause an effect (e.g., incapacitation) in 50 % of the exposed animals.

2.8 ECT_{50}: 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 LC_{50}: 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 LC_{50}, 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 LC_{50} 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 LC_{50}.

2.16 LCT_{50}: 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 LT_{50}: 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 œdema: 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 SO_2 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 irritancy. 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 ED_{50} (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 EC_{50} type of value is most often calculated, for instance the LC_{50} (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 EC_{50} (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 LC_{50} and the EC_{50} are then calculated statistically.

In addition to the commonly quoted LC_{50} and EC_{50} values, two other values are determined: the slope of the concentration-response curve and the confidence limits for the LC_{50} and EC_{50}. The slope of the concentration-response curve is important; for instance, two toxicants with the same LC_{50} may have different effects at a fraction of the LC_{50}. A compound with a steep concentration-response curve would probably have no effect at 0,2 LC_{50}, whereas one with a shallow curve may cause death in 20 % of the animals at 0,2 LC_{50}. In most combustion toxicity experiments the concentration-response curves have been steep. The confidence limits for the LC_{50} describe the certainty with which a given figure lies within a range. The inherent variability of LC_{50} 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 LC_{50} 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 LC_{50}s differ by greater than one order of magnitude. While reported LC_{50}s for individual gases found in fire effluents are distributed over several orders of magnitude, LC_{50}s 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 LC_{50} range, combined with the inherent variability of LC_{50} values derived for fire effluents from materials, exerts an obvious limitation on the numbers of categories into which the LC_{50} 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

relevant biological procedures which have been accepted and validated by the toxicology community. Most appropriately, these methods should have been assessed for their validity. Validation should have included evidence of reproducibility and sensitivity with certain reference toxicological agents such as carbon monoxide, hydrogen cyanide, acrolein and hydrogen chloride. In general, the guidelines for acute inhalation toxicity studies provided by the OECD [2] provide a good basis for a biological test. The biological tests must be performed following the principles of "Good Laboratory Practice" [7,8].

4.2 Fire model

Several different fire models exist. Before any biological assay with animals is performed, the suitability of a fire model must be confirmed. In general, the following criteria should be considered:

— Relevance:

Some relationship must be established between the experimental fire effluent and phases of real fires.

— Definition:

The combustion system and the generation method of fire effluent comprising the fire model must be well defined.

In some cases the equilibrium concentration in an exposure system should be reached in at least one-fifth of the planned exposure time and kept constant thereafter (with a maximum exposure time of 30 min).

The oxygen content of the atmosphere breathed by the animals should not be less than 16 % (V/V) and the temperature not higher than 40 °C for head/nose exposure, but preferably lower, if whole body exposure is used.

In an ideal system, one would expect to determine concentration-response or time-response relationships. Changes in the overall concentration should be made in such a way that the concentration of the different fire effluent is varied proportionally, i.e., the ratio of different compounds in the fire effluent remains constant.

4.3 Analytical measurements

During exposure the concentration of the following fire effluents must be measured (preferably continuously):

— carbon monoxide;

— oxygen;

— carbon dioxide;

— hydrogen cyanide (if present);

— other relevant toxicants, if present (HCl, HBr, etc.).

4.4 Animals

The animals used should either be rats or mice because of the background data available for these species and the availability of established strains. They should be from a known, registered, stable strain and they should be obtained from a reputable breeder. They must be in good health and free of infection, especially pulmonary infection. They must be housed in clean, well-maintained facilities which conform to local regulations or guidelines for the maintenance of laboratory animals. The animals should be humanely treated, in accordance with local guidelines or regulations where appropriate.

The criterion does not preclude the use of other experimental animals in more specialized research in combustion toxicology (e.g., primates for neurological or behavioural studies).

4.5 Experimental design

The philosophy inherent in these criteria is for a study which is designed to assess the acute toxicology of fire effluents as a first evaluation following analytical studies to establish the major putative toxicants. Even though the major toxic effects of fire effluents are narcosis and irritation, the possibility of other effects being produced must be accommodated. For this reason, a group of five male and five female animals should be used for the general acute inhalation toxicity portion of the study (i.e., clinical observations, body weight, *post mortem* examinations). The number of animals required for other specialized investigations such as objective assessment of narcosis or respiratory rate monitoring is left to the judgement of the investigator using a validated method. These animals may be some or all of the animals comprising the general acute toxicity group provided the specialized investigations are not considered to affect the animals adversely. The use of a concurrent, sham-exposed group is recommended to provide a basis for comparison.

The number, sex, strain and species of animals used in any studies, subsequent to the initial general toxicology study should be determined, taking into account the needs of such work.

4.6 "Exposure-Dose"

Most physiological responses are "dose-related", i.e., the magnitude of the response increases with increasing dose or accumulated body burden of a physiologically active agent. Since the actual dose cannot be directly measured in inhalation toxicology, the assumption is made that the accumulated body burden of toxicants from smoke inhalation is a function of smoke concentration and exposure time [9]. As an expression of the insult to which a subject is exposed, the term "Exposure-Dose", is often used.

Concentrations of common fire gas toxicants, such as carbon monoxide (CO) and hydrogen cyanide (HCN) are usually expressed as parts per million (ppm) by volume. Therefore, the Exposure-Dose over a period of time can be expressed as the product of the concentration and time, i.e., in parts per million minutes (ppm·min). In the case of changing concentrations of gaseous toxicant, the Exposure-Dose is actually the integrated area under a concentration *vs.* time curve.

One can also deal with the concept of Exposure-Dose as it applies to fire effluents. Quantification of a fire effluent concentration involves the following parameters:

m_o is the original mass, in milligrams, of sample;

m_r is the mass, in milligrams, of the residue of the sample after test;

q_V is the rate flow of air, in litres per minute, into the exposure chamber for dynamic, steady-state systems;

V is the volume of air, in litres, in the chamber for constant volume systems;

t is the duration of exposure, in minutes.

The following can be determined from the above values to describe the amount of material used in a study:

Nominal furnace load concentration:

$$C_{FL} = \frac{m_o}{q_V \cdot t} \text{ or } \frac{m_o}{V}$$

Nominal mass loss concentration:

$$C_{WL} = \frac{m_o - m_r}{q_V \cdot t} \text{ or } \frac{m_o - m_r}{V}$$

These expressions indicate the concentration of fire effluents which would be present if either the mass of the test material used or the mass of the material consumed were to be distributed uniformly into the diluting air. The calculated values should be correlated with data from adequate monitoring methods to determine the actual concentrations of fire effluents.

Especially in "static systems", the nominal mass loss concentration may be misleading where the thermal decomposition process lasts for a significant portion of the animal exposure time. During this time the fire effluent concentrations increase and steady concentrations of effluents in terms of either toxic products or amount of material consumed are not reached until late into the exposure. In these cases, in which the thermal decomposition process lasts for longer than 5 % of the exposure period, the consumption of the test material must be monitored by mass loss at least every minute.

Once fire effluent concentration has been calculated, the Exposure-Dose can be determined in a manner quite analogous to that used for single gaseous toxicants. Expressed in milligrams minutes per litre, the Exposure-Dose is the integrated area under the fire effluent concentration *vs.* time plot associated with an exposure [8].

In practice, the Exposure-Dose is often calculated by summation of incremental mass loss values per unit volume of the system, multiplied by the incremental time associated with the mass loss. For a static/constant volume system, this would be expressed as follows:

$$\text{Exposure-Dose} = \sum_{i=1}^{n} \frac{(\Delta m)_i \cdot (\Delta t)_i}{V}$$

where

Δm is the incremental mass loss, in milligrams, over Δt;

Δt is the incremental time, in minutes;

V is the dilution volume, in litres, of the system;

n is the number of incremental time segments employed.

For the calculation in the case of a dynamic/steady-state system:

$$\text{Exposure-Dose} = \sum_{i=1}^{n} \frac{(\Delta m)_i}{q_V}$$

where q_V is the flow rate, in litres per minute.

For situations in which material mass loss is not determined incrementally and thermal degradation can be ascertained to be at a uniform rate, Δm in the above equations is the material mass loss occurring over the total time of the exposure.

The use of the Exposure-Dose concept is recommended to accommodate potential anomalies aris-

ing from the occurrence of a slow rate of thermal degradation in a static/constant volume system, or a non-steady-state thermal degradation in a dynamic system.

4.7 Exposure duration

The relationship between time and concentration is important in combustion toxicology. In order to provide some data on this relationship, two exposure time periods of 5 min and 30 min should be used for each furnace condition.

If LT_{50} values need to be determined, other exposure time periods can be used. In any event, the exposure time should be long enough to guarantee chamber equilibrium.

4.8 Thermal decomposition methods and exposure methods

The term "dynamic" has been applied for exposure methods in which the fire effluents are generated continuously (dynamic) over the total exposure time period using a flow-through system. This is intended to result in a "steady-state" concentration profile in the exposure chamber, if the decomposition conditions are kept unchanged. If the decomposition conditions are changed during the experiment, a "non-steady-state" concentration profile will result.

The term "static" has been applied for exposure methods by which the fire effluents are generated in a defined volume of air. Therefore, alternatively, the term "constant volume" (i.e., closed system) can be used for this method, resulting in a "non-steady-state"concentration profile. A "steady-state" concentration profile may be reached after the end of the decomposition process and proper mixing in the chamber, if absorption on to chamber surfaces can be neglected.

It may sometimes be an advantage to use both methods of exposure, if required (e.g., relevance to certain fire situations). The following basic requirements are considered to be essential for the selection of a suitable exposure method:

a) the exposure method which enables good repeatability and reproducibility of test results should be selected

b) significant heat stress or oxygen depletion should be avoided;

c) it must be possible to determine concentration-response or time-response relationships. Changes in concentration should be obtained so that the ratio of different components of the fire effluent remains constant.

4.9 Animal exposure modes

Two different exposure modes may be used:

— whole body exposure modes (animals in chambers);

— head-nose exposure modes (animals in tubes).

The head-nose exposure mode has been used predominantly.

Some basic requirements are considered to be essential for both

a) the materials used to build the exposure apparatus should be as inert and non adsorbent as possible and should allow good observation of the animals;

b) the exposure chamber should provide for homogeneous distribution of fire effluents and rapid filling;

c) The environmental conditions (temperature, pressure, humidity, air-flow and velocity) as well as the maximum tolerable number of animals per unit of chamber volume should be in accordance with international guidelines and published experience [2, 3, 10], to allow for reproducibility of the tests and to avoid unnecessary stress or discomfort to the animals.

4.10 Observations and examinations

The animals should be observed

a) during exposure;

b) immediately post-exposure;

c) and daily for at least a 14-d post-exposure period.

4.10.1 Observations during exposure

The animals must be observed throughout the exposure whenever visibility permits. Particular attention should be directed to

— observations of breathing behaviour, motor activity (e.g., lethargy, coma);

— changes of mucous membranes (e.g., salivation);

— effects on central nervous system (e.g., tremor, convulsions).

A method for the objective assessment of narcosis should be used.

Particular attention should be given to irritant effects such as changes in respiratory pattern and nasal discharge. Time of death should be recorded as precisely as possible.

4.10.2 Observations immediately post-exposure

All animals, including controls, must be subjected to a clinical examination as soon after the exposure terminates as practicable. This evaluation should include, but not be limited to, changes in the skin and fur (condition of pelt), eyes (condition of eyes, corneal reflex), mucous membranes (conditions, salivation) respiratory, circulatory, autonomic and central nervous system (pinna reflex, foot withdrawal reflex) and somato-motor activity (righting reflex). Particular attention should be directed to observation of tremors, convulsions, diarrhoea, lethargy, sleep and coma. The time of death should be recorded as precisely as possible. Measurements of COHb on animals dying during exposure, or on survivors within 5 min of exposure, should be made if an establishment of the toxicity related to CO is essential. Further measurements (blood cyanide, thiocyanate in urine) should be considered.

4.10.3 During the observation period

Surviving animals must be maintained for a 14-d post-exposure period. During this time they must be clinically examined daily. Their body mass should be recorded before exposure and 1 d, 2 d, 3 d, 7 d and 14 d after exposure.

4.11 *Post mortem* examination

Any animal dying must be subjected to a *post mortem* examination, except where autolysis would make this valueless. An additional group of animals should be killed and examined 48 h after exposure if oedema is to be assessed. All animals should be killed at the end of the 14-d post-exposure observation period. The examination should include assessment of the major abdominal and thoracic organs, and the following organs should be removed, trimmed and weighed: lungs, liver, kidney. Selected tissues may be retained for possible histopathological evaluation.

4.12 Results, data and reporting

The results of the studies should be presented so that the quantitative and qualitative aspects of the toxicity of the fire effluents can be determined. This will involve tabulation of the results, the use of statistical evaluation methods where appropriate and interpretation of the data.

Data may be summarized in tabular form showing for each test group the number of animals at the start of the test, time of death or exposure time periods of individual animals at different exposure levels, number of animals displaying other signs of toxicity, description of toxic effects and necropsy findings. Quantitative values such as LC_{50} or EC_{50} may be determined by any appropriate published statistical method.

Any quantitative values such as LC_{50} should be considered in conjunction with the observed specific toxic effects and the necropsy findings. Reference should always be made to the experimental animal species and the exposure time. An evaluation should include the relationship, if any, between the animals' exposure to fire effluents and the incidence and severity of all abnormalities, including behavioural and clinical abnormalities, gross lesions, body mass changes, mortality and other toxic effects.

The test report should include the following information:

a) *Test conditions*

- methods and conditions of fire effluent generation;
- samples used for generation of fire effluents (material, volume and/or mass configuration);
- description of mode of exposure, exposure systems, including design, type, dimensions;
- description of the equipment for measuring temperature, air flow, and gas concentrations.

b) *Exposure data*

- airflow rates through the inhalation equipment;
- temperature in the breathing zone of the animals;
- the definition of the concentration should be given [nominal furnace load concentration (C_{FL}) or nominal mass loss concentration (C_{WL})]. Values reported in correlation with an effect should be given in the following way:

  $M\ EC_x = \ldots$ mg/l $(t, C_{FL}$ or $C_{WL}, T, FM, O)$

  or

  $M\ LC_x = \ldots$ mg/l $(t, C_{FL}$ or $C_{WL}, T, FM, O)$;

- the experimental conditions (duration of exposure = t, decomposition temperature = T, fire model = FM) must be always written in parentheses together with the quantitative value of the material M.

M = material;

EC = effective concentration (or LC);

x = percentage of animals responding;

t = duration of exposure;

T = decomposition temperature;

O = duration of observation period over which effect is scored;

FM = fire model used;

mg/l = nominal furnace load concentration C_{FL} or nominal mass loss concentration (C_{WL});

— the Exposure-Dose could be reported in milligrams minutes per litre;

— oxygen concentration in the inhalation atmosphere;

— analytical measured concentrations of toxicants (e.g., CO, HCN, CO_2, HCl, etc.).

c) *Animal data*

— species/strain used;

— tabulation of response data by sex and exposure levels (i.e., number of animals showing signs of toxicity, number of animals exposed);

— time of death during or following exposure;

— toxicity values (LC_{50}, EC_{50}, etc.) for each sex determined at the end of the observation period (with method of calculation specified);

— Exposure-Dose lethality curve and slope (where permitted by the method of determination);

— necropsy and histopathological findings including a record of lesions and abnormalities observed;

— test results should be compared with results from appropriate reference compounds (such as CO, HCN, irritants), performed in the same laboratory.

5 Recommendations of methodology

Much of the methodology appropriate for biological assays of the acute inhalation toxicity of fire effluents was developed for and has been utilized in research and development of various chemicals, drugs, pesticides, etc. [2, 3, 4]. It has been shown by intensive research, that these methods can also be applied to the assessment of the toxicity of fire effluents.

The methodology has been extensively reviewed [6] and several methods are available which meet the principles and criteria set forth in clause 3 and clause 4. These methods are therefore suitable to assess the acute inhalation toxicity of fire effluents, but no recommendations are given for a general preference of one or two specific methods. It is left to the responsibility of qualified toxicologists to select one or more methods suitable for the assessment of toxicity on a case-by-case basis.