
**Estimation of the lethal toxic potency
of fire effluents**

Détermination du pouvoir toxique létal des effluents du feu

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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.

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.

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 13344 was prepared by Technical Committee ISO/TC 92, *Fire safety*, Subcommittee SC 3, *Fire threat to people and environment*.

This second edition cancels and replaces the first edition (ISO 13344:1996), which has been technically revised.

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Introduction

The pyrolysis or combustion of every combustible material produces a fire effluent atmosphere, which, in sufficiently high concentration, is toxic. It is, therefore, desirable to establish a standard test method for the determination of the toxic potency of such fire effluents.

It is further desirable, in view of worldwide resistance to the exposure of animals in standard tests, that this method should not make mandatory the use of such animals in its procedures. The mandatory portion of this standard test does not, therefore, specify the use of animal exposures. It only refers to animal exposure data already reported in the literature, with calculations being employed to express test results as they would have been obtained had animals actually been employed.

For those cases in which confirmation of test results using animal exposures can be justifiably permitted, an optional procedure to do so is presented in Annex A.

The two parameters calculated using this standard are the FED (Fractional Effective Dose) and the LC_{50} . When either of these is used in performing a hazard analysis, certain information must accompany the term to avoid confusion. In the case of the FED, that is the toxicological effect on which the FED is based and the species for which the FED has been determined. In the case of the LC_{50} , that information is the length of the exposure and the animal species for which the LC_{50} has been determined.

Estimation of the lethal toxic potency of fire effluents

1 Scope

This International Standard provides a means for estimating the lethal toxic potency of the fire effluents produced from a material while exposed to the specific combustion conditions of a physical fire model. The lethal toxic potency values are specifically related to the fire model selected, the exposure scenario and the material evaluated.

Lethal toxic potency values associated with 30-min exposures of rats are predicted using calculations which employ combustion atmosphere analytical data for carbon monoxide (CO), carbon dioxide (CO₂), oxygen (O₂) (vitiation) and, if present, hydrogen cyanide (HCN), hydrogen chloride (HCl), hydrogen bromide (HBr), hydrogen fluoride (HF), sulfur dioxide (SO₂), nitrogen dioxide (NO₂), acrolein and formaldehyde. If the fire effluent toxic potency cannot be attributed to the toxicants analysed, this is an indication that other toxicants or factors must be considered.

This International Standard is applicable to the estimation of the lethal toxic potency of fire effluent atmospheres produced from materials, products or assemblies under controlled laboratory conditions and should not be used in isolation to describe or appraise the toxic hazard or risk of materials, products or assemblies under actual fire conditions. However, results of this test may be used as elements of a fire hazard assessment that takes into account all of the factors which are pertinent to an assessment of the fire hazard of a particular end use; see ISO/TS 19706.

The intended use of fire safety-engineering calculations is for life-safety prediction for people and is most frequently for time intervals somewhat shorter than 30 min. This extrapolation across species and exposure intervals is outside the scope of this International Standard.

This International Standard does not purport to address all of the safety problems associated with its use. It is the responsibility of the user of this International Standard to establish appropriate safety and health practices.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO/TS 13571:2002, *Life-threatening components of fires — Guidelines for the estimation of time available for escape using fire data*

ISO 13943:2000, *Fire safety — Vocabulary*

ISO 19701—¹⁾, *Fire safety — Sampling and analytical methods for fire effluents*

ISO/TS 19706, *Guidelines for assessing the fire threat to people*

1) To be published.

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 13943:2000 and the following apply

3.1

carboxyhaemoglobin saturation

percentage of blood haemoglobin converted to carboxyhaemoglobin from the reversible reaction with inhaled carbon monoxide

3.2

concentration-time curve

plot of the concentration of a gaseous toxicant or fire effluent as a function of time

NOTE The typical units for the concentration of a toxic gas are $\mu\text{l/l}$ and, for fire effluent, $\text{g}\cdot\text{m}^{-3}$. The units of $\mu\text{l/l}$ are numerically identical to ppm by volume, the use of which is discouraged.

3.3

exposure dose

measure of a gaseous toxicant or of a fire effluent available for inhalation, calculated by integration of the area under a concentration-time curve

NOTE The typical units are $\mu\text{l}\cdot\text{l}^{-1}\cdot\text{min}$ for a gaseous toxicant and $\text{g}\cdot\text{m}^{-3}\cdot\text{min}$ for fire effluent. The units of $\mu\text{l/l}$ are numerically identical to ppm by volume, the use of which is discouraged.

3.4

fractional effective dose

FED

ratio of the exposure dose for an asphyxiant toxicant to that exposure dose of the asphyxiant expected to produce a specified effect on an exposed subject of average susceptibility

NOTE 1 As a concept, FED refers to any effect, including incapacitation, lethality or other endpoints.

NOTE 2 Values of FED that are based on lethality are designated as L_{FED} , while those based on incapacitation are designated as I_{FED} .

NOTE 3 When not used with reference to a specific asphyxiant, the term FED represents the summation of FED values for all asphyxiants in a combustion atmosphere.

3.5

lethal concentration 50

LC_{50}

concentration of a toxic gas or fire effluent statistically calculated from concentration-response data that causes death in 50 % of a population of a given species within a specified exposure and post-exposure time

NOTE The typical units are $\mu\text{l/l}$ for a gaseous toxicant and $\text{g}\cdot\text{m}^{-3}$ for fire effluent. The units of $\mu\text{l/l}$ are numerically identical to ppm by volume, the use of which is discouraged.

3.6

mass charge concentration

mass of a test sample placed in a combustion chamber per unit chamber volume (for a closed system) or per total volume of air passing through an open system

NOTE 1 The typical units are $\text{g}\cdot\text{m}^{-3}$.

NOTE 2 For an open system, the definition assumes that the mass is dispersed in the airflow uniformly over time.

3.7

mass loss concentration

mass of a test sample consumed during combustion per unit chamber volume (for a closed system) or per total air flow passing through an open system

NOTE 1 The typical units are $\text{g}\cdot\text{m}^{-3}$.

NOTE 2 For an open system, the definition assumes that the mass is dispersed in the airflow uniformly over time.

3.8

predicted LC_{50}

LC_{50} for the effluent of a combusted test sample, calculated from combustion atmosphere analytical data, as that effluent concentration that would yield an FED equal to 1

NOTE The typical units are $\mu\text{l/l}$ for a gaseous toxicant and $\text{g}\cdot\text{m}^{-3}$ for fire effluent. The units of $\mu\text{l/l}$ are numerically identical to ppm by volume, the use of which is discouraged.

4 Principle

4.1 This method subjects a test sample to the combustion conditions of a specific physical fire model.

Concentrations of the major gaseous toxicants in the fire effluent atmosphere are monitored over a 30-min period, with $(C\cdot t)$ products for each interval being determined from integration of the areas under the respective concentration vs time plots. The $(C\cdot t)$ product data, along with either the mass charge or the mass loss of the test sample during the test, are then used in calculations to predict the 30-min LC_{50} of the test sample.

4.2 Since there can be toxicants present other than those measured, this value of the LC_{50} is a maximum.

If the chemical formulation and professional experience suggest that additional toxicants might contribute significantly to the LC_{50} value, the accuracy of the predicted LC_{50} may then be experimentally determined using a bioassay (see Annex A). Agreement within the experimental uncertainty supports attributing the lethality of the smoke to the monitored toxicants.

4.3 The strategy employed in this method for quantification of fire effluent toxic potency represents utilization of the latest in state-of-the-art understanding of the prediction of the toxic effects of fire effluents as reported in ISO/TR 9122-5[2].

Toxic potencies are calculated from combustion product analytical data without the exposure of experimental animals. Such a methodology is based on extensive experimentation using exposure of rats to the common fire gases, both singly and in combinations; see Reference [2]. The principal can be expressed mathematically, as shown in Equation (1); see Reference [3]:

$$L_{\text{FED}} = \sum_{i=1}^n \int_0^t \frac{C_i}{(C\cdot t)_i} dt \quad (1)$$

where

C_i is the concentration, expressed in microlitres per litre, of the toxic component, i ;

$(C\cdot t)_i$ is the concentration-time product, expressed in microlitres per litre times minutes, for the specific exposure doses required to produce the toxicological effect.

When, as in this test method, the time values of 30 min numerically cancel, the FED becomes simply the ratio of the average concentration of a gaseous toxicant to its LC_{50} value for the same exposure time. When the FED is equal to 1, the mixture of gaseous toxicants should be lethal to 50 % of exposed animals.

5 Significance and use

5.1 This test method has been designed to provide data for use in the assessment of lethal toxic fire hazard as a means for the evaluation of materials and products and to assist in their research and development.

The data are not, in themselves, an indication of toxic hazard or relative toxic hazard of a commercial product.

5.2 The method is used to predict the LC_{50} of fire effluents produced upon exposure of a material or product to fire.

Experimental confirmation might be needed to determine whether the major gaseous toxicants can account for the observed toxic effects as well as for the lethal toxic potency (see Annex A).

5.3 Predicted LC_{50} values determined in this test method are associated only with the physical fire model used.

5.4 This test method does not attempt to address the toxicological significance of changes in particulate/aerosol size, fire effluent transport, distribution or deposition, or changes in the concentration of any fire effluent constituent as a function of time as may occur in a real fire.

5.5 The propensity for fire effluents from any material to have the same effects on humans as on rats in fire situations can only be inferred to the extent that the biological system of the rat is correlated with the human system.

5.6 This test method does not address any other acute sublethal effects of smoke, e.g., sensory and upper-respiratory-tract irritation, reduced motor capability, heat or thermal radiation injury, etc.

5.7 This test method does not address the long-term lethal effects of smoke exposure or the lethal effects of chronic exposures to smoke.

5.8 The FED values, L_{FED} , estimated from this method differ from those obtained using the equations in ISO/TS 13571. The values obtained here are derived from rat lethality data. The FED values from ISO/TS 13571 are derived from consensus estimates of the incapacitating effects of fire gases on people.

6 Apparatus

6.1 Physical fire model

6.1.1 The physical fire model, or laboratory combustion device, and the conditions under which it is operated shall be chosen so as to have demonstrated relevance to one or more of the specific classes or stages of fires identified and characterized in ISO/TS 19706.

6.1.2 When obtaining data on the effluent from the combustion of a commercial product or assembly, i.e., other than a homogeneous material, the configuration and condition of the test sample in the physical fire model shall be relevant to the appropriate fire exposure of the commercial product or assembly.

6.1.3 Repeatability and inter-laboratory reproducibility of the physical fire model shall be demonstrated to be within the uncertainty range for the FED calculations for irritant and asphyxiant gases in ISO/TS 13571.

6.1.4 The physical fire model shall be adaptable to analytical requirements.

6.2 Gas sampling

6.2.1 Continuous gas sampling shall be used to measure CO , CO_2 and O_2 levels.

6.2.2 The gas analysers shall have the following ranges, as a minimum:

- carbon monoxide, 0 % to 1 % (0 µl/l to 10 000 µl/l);
- carbon dioxide, 0 % to 10 %;
- oxygen, 0 % to 21 %.

6.2.3 Other gas analyses (for example, for HCN, HCl, HBr, NO_x, SO₂, acrolein, formaldehyde and other chemical species) shall be performed, as appropriate to the chemical composition of the test sample and/or expectation of potential combustion products, by a method of choice with guidance from ISO 19701.

7 Hazards

7.1 This test procedure involves combustion processes.

Therefore, hazards to operating personnel exist from inhalation of combustion products. To avoid accidental leakage of toxic combustion products into the surrounding atmosphere, the entire exposure system shall be placed in a laboratory fume hood or under a canopy hood.

7.2 The venting system shall be checked for proper operation before testing and shall discharge into an exhaust system with adequate capacity.

7.3 Operating personnel have the responsibility to assure that they are in compliance with all pertinent regulations regarding release and/or disposal of combustion products or gases.

8 Test samples

8.1 Test samples shall be prepared in accordance with the operating restrictions and conditions applicable to the physical fire model used and with consideration of the end use of the finished product being examined.

8.2 Test samples shall be conditioned at an ambient temperature of 23 °C ± 3 °C (73 °F ± 5 °F) and relative humidity of (50 ± 10) % for at least 24 h prior to testing or until constant weight is attained.

9 Calibration of the apparatus

9.1 Physical fire model calibrations shall be conducted in accordance with the applicable operating methodology of the physical fire model.

9.2 Gas analyser calibrations shall be conducted at the beginning of each series of tests.

9.2.1 The gas analysers (for O₂, CO₂, and CO) shall be calibrated using nitrogen gas for “zeroing” and an appropriate gas mixture close to, but less than, the analyser full-scale reading.

For all calibrations, the gas shall be set to flow at the same rate and pressure as the sample gas. For calibration of the O₂ analyser, ambient air (20,9 % O₂ if the air is dry) shall be used, while for the CO₂ and CO analysers, bottled gases containing CO₂ or CO at known concentration are required. A single mixture containing both CO and CO₂ may be used. During the calibration procedure the gas return lines shall be diverted into an exhaust duct in order to prevent inadvertent accumulation of CO and CO₂ in the exposure chamber.

9.2.2 Calibration of devices used for analysis of other gases (for example, HCN, HCl and HBr) shall be performed using the guidance provided in ISO 19701.

10 Procedures

10.1 General

10.1.1 The test conditions in the physical fire model shall replicate the combustion conditions in the intended fire stage.

10.1.2 The choice of sample size for initial tests is made with consideration of anticipated toxicant yields such that L_{FED} s from 0,7 to 1,3 are obtained (see Clause 11) over the 30-min test period. Analytical data from at least three tests are used for the calculation of a predicted LC_{50} for the test sample (Clause 12) to test for possible sensitivity to sample size of combustion conditions in the test apparatus.

10.2 Preparation for tests

Test preparation shall be conducted in accordance with the operating procedures for the physical fire model.

10.3 Test procedure for obtaining data

10.3.1 Weigh the conditioned test sample and subject it to the operating conditions of the physical fire model.

10.3.2 As specified in Clause 12, collect analytical data for a total of 30 min from the initiation of the test or from when the combustion conditions replicating the desired fire stage (6.1.1) are established within the apparatus.

10.3.3 Quench the test sample residue, remove it from the sample holder, and cool it to ambient temperature in an exhaust hood.

Weigh the sample residue after it has cooled. Use reasonable means to obtain an accurate measure of the mass of the test sample that has not been combusted, recognizing that some samples can lose material from the sample holder, for example, by explosion or spitting.

11 Calculations

11.1 General

The predicted lethal toxic potency (LC_{50}) of the test sample is calculated from the combustion atmosphere analytical data for CO, CO₂, O₂, and, if present, HCN, HCl and other toxicants. This is done for a given sample mass by first calculating the FED for the test. The LC_{50} is then calculated as that sample mass which would yield an FED equal to 1 within a volume of 1 m³.

11.2 Calculation of FED

11.2.1 Two equations have been developed for the estimation of the 30-min lethality FED from the chemical composition of the environment in the physical fire model. Each begins with the precept that the fractional lethal doses of most gases are additive, as developed by Tsuchiya and co-workers^[4].

11.2.2 Equation (2) was developed empirically by Levin and co-workers^[5] from exposure of laboratory rats to individual and mixed gases

$$L_{FED} = \frac{m[CO]}{[CO_2] - b} + \frac{21 - [O_2]}{21 - LC_{50,O_2}} + \frac{[HCN]}{LC_{50,HCN}} + \frac{[HCl]}{LC_{50,HCl}} + \frac{[HBr]}{LC_{50,HBr}} \quad (2)$$

which reduces to

$$L_{FED} = \frac{m[CO]}{[CO_2] - b} + \frac{21 - [O_2]}{(21 - 5,4)} + \frac{[HCN]}{150} + \frac{[HCl]}{3\,700} + \frac{[HBr]}{3\,000}$$

where

m	is the slope of the CO-vs-CO ₂ curve, which depicts the increasing toxicity of CO as CO ₂ concentration increases;
b	is the intercept of the CO-vs-CO ₂ curve, which depicts the increasing toxicity of CO as CO ₂ concentration increases;
[O ₂]	is the O ₂ concentration, expressed in percent by volume ;
[HCN]	is the HCN concentration, expressed in microlitres per litre;
[HCl]	is the HCl concentration, expressed in microlitres per litre;
[HBr]	is the HBr concentration, expressed in microlitres per litre;
[CO]	is the CO concentration, expressed in microlitres per litre;
[CO ₂]	is the CO ₂ concentration, expressed in percent by volume;
LC _{50,HCN}	is the LC ₅₀ for HCN, expressed in microlitres per litre;
LC _{50,HCl}	is the LC ₅₀ for HCl, expressed in microlitres per litre;
LC _{50,HBr}	is the LC ₅₀ for HBr, expressed in microlitres per litre.

The values of m and b depend on the concentration of CO₂. If [CO₂] ≤ 5 %, $m = -18$ and $b = 122\,000$. If [CO₂] > 5 %, $m = 23$ and $b = -38\,600$. Confirmatory work using this model has been published by Pauluhn^[6].

NOTE 1 The values of all gas concentrations are the integrated ($C \cdot t$) product values taken from their respective concentration-time curves over the 30-min test period divided by 30. For each individual toxicant, the LC₅₀ values are those that have been statistically determined from independent experimental data to produce lethality in 50 % of test animals (rats) within a 30-min exposure plus a 14-day post-exposure period.

NOTE 2 This concept that the toxic potency of smoke may be approximated by the contributions of a small number of gases has been termed the "N-Gas Model" by the U.S. National Institute of Standards and Technology (NIST). The "N-Gas Model" takes into account the effects of CO₂ on the toxicity of CO, as expressed empirically from rat exposure studies conducted at NIST. Equation (2) also takes into consideration oxygen vitiation in the event that it is significant. Examination of a series of pure gaseous toxicant experiments in which various percentages of rats died indicated that the mean FED value using the "N-Gas" calculation was 1,07 where one-half of the test animals died. The 95 % confidence interval was 0,25; see Reference [7].

11.2.3 Equation (3) was developed by Purser^[8], fitting the rat LC₅₀ data obtained mainly by Levin *et al.*^[5] and Kaplan and Hartzell^[9].

$$L_{\text{FED}} = \left(\frac{[\text{CO}]}{\text{LC}_{50,\text{CO}}} + \frac{[\text{CN}]}{\text{LC}_{50,\text{HCN}}} + \frac{[\text{X}]}{\text{LC}_{50,\text{X}}} + \frac{[\text{Y}]}{\text{LC}_{50,\text{Y}}} \right) \times V_{\text{CO}_2} + Z_{\text{A}} + \frac{21 - [\text{O}_2]}{21 - 5,4} \quad (3)$$

where

[CN]	is the HCN concentration, expressed in microlitres per litre, corrected for the presence of other nitriles and the protective effect of NO ₂ , and is equal to [HCN] plus [total organic nitriles] minus [NO ₂];
[X]	is the concentration, expressed in microlitres per litre, of each acid gas;
[Y]	is the concentration, expressed in microlitres per litre, of each organic irritant;
LC _{50,X}	is the LC ₅₀ , expressed in microlitres per litre, of each acid gas irritant;

$LC_{50,y}$ is the LC_{50} , expressed in microlitres per litre, of each organic irritant;

$[CO_2]$ is the CO_2 concentration, expressed in percent by volume;

V_{CO_2} is a multiplication factor for CO_2 -driven hyperventilation, equal to $1 + e[(0,14 \cdot CO_2) - 1]/2$;

Z_A is an acidosis factor, equal to $[CO_2] \times 0,05$.

The 30-min LC_{50} values (from ISO/TR 9122-5^[2]) used in Equation (3) are given in Table 1.

Table 1 — 30 min LC_{50} values for rats

Fire effluent gas	30-min LC_{50} $\mu\text{l/l}$
CO	5 700
HCN	165
HCl	3 800
HBr	3 800
HF	2 900
SO ₂	1 400
NO ₂	170
Acrolein	150
Formaldehyde	750

NOTE 1 Equations (2) and (3) give different FED values for a given set of input gas concentrations. There are small numerical differences in the LC_{50} values for HCN, HCl, and HBr. These differences are within the uncertainties of the experiments from which the values in 11.2.2 were derived. No confidence limits are available for Equation (3).

NOTE 2 There are functional form differences in the way $[CO]$, $[CO_2]$ and depleted O_2 are included in Equations (2) and (3). Thus, there will be differences in the values of the FED calculated using the two equations. For well-ventilated, pre-flashover fires ($[CO_2] < 3\%$ by volume and $[CO]/[CO_2] < 0,1$), the differences in calculated FED are within $\pm 20\%$. As the ventilation becomes more limited, the FED values, as well as these differences, increase, for example approaching a factor of 2 for $[CO_2] = 10\%$ by volume and $[CO]/[CO_2] = 0,5$. Since FED values $\gg 1$ are not compatible with survival for times near 30-min, a reasonable estimate of agreement among the two equation calculations is $\pm 30\%$ for FED values ≈ 1 .

11.3 Calculation of predicted LC_{50}

11.3.1 The predicted 30-min LC_{50} for each test sample in a series of tests is calculated from Equation (4):

$$LC_{50} = \frac{M}{L_{FED} \times V} \quad (4)$$

where

M is the specific mass loss, in grams;

V is the total air volume, in cubic metres at standard temperature and pressure. For a closed system, this is the contained volume of the apparatus; for an open (flow-through) system, this is the total flow during the combustion of the test sample.

The resulting predicted LC_{50} are expressed in grams per cubic metre.

FED values used in Equation (4) should be between 0,5 and 1,5 in order to minimize extrapolation errors introduced from using toxic gas concentrations that are exceedingly low or high.

11.3.2 Prior experience has shown that the accuracy of LC_{50} values determined in this manner is $\pm 30\%$ if the concentrations of all the contributing toxicants are measured and included.

However, if toxicants in addition to those included in Equations (2) and (3) are present, then the uncertainty must be determined specifically for that sample, such as by using a bioassay as described in Annex A.

12 Test report

12.1 The report shall provide the following information for each test in a series:

- a) name and address of the testing laboratory;
- b) names of responsible persons at the testing laboratory;
- c) test identification and date;
- d) laboratory ambient conditions (temperature and humidity);
- e) description of sample, including rationale for its configuration and condition relative to the end use of the product being examined;
- f) physical fire model and conditions of operation, including documented evidence concerning the relevance of the chosen model;
- g) mean exposure chamber temperature;
- h) maximum exposure chamber temperature and time when attained;
- i) initial sample mass and mass loss during test, expressed in grams per cubic metre of air volume, including any observations of mass lost from the sample holder by processes other than combustion;
- j) observations of sample including melting, char formation, spalling, unusually vigorous burning and re-ignition;
- k) gas analysis data, including integrated $(C \cdot t)$ product values over the 30-min test for the toxicants analysed, minimum O_2 concentration and maximum CO_2 concentration, times to reach minimum O_2 and maximum CO_2 . The methods used for analyses should be identified;
- l) calculation of:
 - $(C \cdot t)$ product for each analysed toxicant,
 - $(C \cdot t)$ product for each analysed toxicant divided by 30 min,
 - indication whether Equation (2) or Equation (3) was used,
 - FED for each test,
 - predicted LC_{50} , specifying the calculation method used;
- m) optionally, plots of individual toxicant concentrations, sample mass loss and temperature as functions of time.

12.2 The test report shall provide a best predicted LC_{50} value calculated from the results of all tests conducted.

This is accomplished from a linear regression analysis of a plot of sample mass versus FED value. The mass value corresponding to an FED equal to 1 is then used in Equation (4) to calculate the best predicted LC_{50} value.

13 Precision and bias

13.1 The precision of the lethal toxic potency value from each physical fire model shall be determined individually. The estimated agreement of the LC_{50} values (derived from a single data set of gas concentrations) using Equations (2), (3) and (4) is $\pm 30\%$.

13.2 The bias of this test method has not been measured since there is no accepted reference material for use in making such measurements.

NOTE The (U.S.) National Institute of Standards and Technology maintains a Standard Reference Material for use in lethal toxic potency estimations. LC_{50} values have not been established for all current physical fire models.

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Annex A (informative)

Optional bioassay for confirmation of predicted LC₅₀ values

A.1 General

A predicted LC₅₀ may be experimentally confirmed, supporting attribution of the lethality of the smoke to the monitored toxicants. The potential use of animal exposures to confirm a predicted LC₅₀ value is intended to involve broad discretion for such a decision by professionals qualified by education and experience to do so. The decision to expose animals must be defensible in view of both the need of the information to be gained and its value to human safety. The risk of not performing animal exposures is that the toxic potency will be underestimated due to the presence of an unknown toxicant. This underestimation is likely to be low for small changes in product formulation and potentially higher for new polymers or new polymer-additive combinations. Thus, the use of animal exposures can and should be minimized or even avoided except in those cases where professional judgment indicates significant consequences for not performing the confirming tests.

It is the responsibility of the user to establish appropriate practices and determine the applicability of regulatory limitations, particularly with regard to the care and use of experimental animals, prior to use. Experimental confirmation of predicted LC₅₀ values must also comply with good laboratory practice regulations (see References [10] and [11]) to assure the quality and integrity of data obtained and adhere to applicable regulations with regard to the care and use of experimental animals. General guidance is also offered in ISO/TR 9122-2^[1].

A.2 General guidance

A.2.1 Test animals shall be inbred, healthy, young adult, male or female rats; (see A.3.2 for the use of mice).

The rats shall be obtained from a reputable supplier that certifies its animals to be specific pathogen-free.

A.2.2 Maintenance and care of animals shall be performed by qualified personnel in accordance with relevant guidelines.

The animal housing facilities shall be suitable to studies of this type.

A.2.3 Upon receipt, the animals shall be identified, weighed and housed in a separate quarantine area for a minimum of five days prior to testing.

Cage assignments shall be made according to a randomization routine. During the quarantine period, animals shall be observed regularly. Animals that are unsuitable by reason of size, health or other criteria shall not be used. Weight gain between time of arrival and before testing is a good indicator of health.

A.2.4 The animals should preferably be housed one to a cage. If this is not feasible, provision must be made for proper animal identification.

The environment shall have proper ventilation and be controlled to a temperature of 23 °C ± 3 °C (73 °F ± 5 °F) and have a relative humidity of (50 ± 15) %. The animal room shall have a 12-h light/dark cycle.

A.2.5 Animal observations, determination of body weight and sacrifice of animals must be performed in accordance with applicable recognized guidelines, such as the OECD Guidelines for Testing of Chemicals^[10] and the Good Laboratory Practice Regulations^[11].