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Estimation of the lethal toxic potency of fire effluents

Détermination du pouvoir toxique létal des effluents du feu

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Contents

Page

Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 92, *Fire safety*, Subcommittee SC 3, *Fire threat to people and environment*.

This third edition cancels and replaces the second edition (ISO 13344:2004), which has been technically revised. The following changes have been made:

— ISO 19702 has been added as a normative reference and citations added in 6.2.3 and 9.2.2;

— the first paragraph in 4.3 has been deleted;

— the note in 13.2 has been deleted.

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 estimation 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 animal 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 effluent 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 (CO_2), oxygen (O_2) (vitiation) and, if present, hydrogen cyanide (HCN), hydrogen chloride (HCl), hydrogen bromide (HBr), hydrogen fluoride (HF), sulfur dioxide (SO_2), nitrogen dioxide (NO_2), acrolein and formaldehyde. The chemical composition of the test specimen may suggest additional combustion products to be quantified and included. If the fire effluent toxic potency cannot be attributed to the toxicants analysed (Annex A), 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 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 13571, *Life-threatening components of fire — Guidelines for the estimation of time to compromised tenability in fires*

ISO 13943:2008, *Fire safety — Vocabulary*

ISO 19701, *Methods for sampling and analysis of fire effluents*

ISO 19702, *Guidance for sampling and analysis of toxic gases and vapours in fire effluents using Fourier transform infrared (FTIR) spectroscopy*

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

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 13943:2008 apply.

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 ($C·t$) products for each interval being determined from integration of the areas under the respective concentration vs time plots. The ($C·t$) 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 Toxic potencies are estimated 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[1]. The principle can be expressed mathematically, as shown in Formula (1); see Reference[2]:

$$L_{\text{FED}} = \sum_{i=1}^{n} \int_{0}^{t} \frac{C_i}{\left(C \cdot t\right)_i} \text{d}t \tag{1}$$

where

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

$(C·t)_i$ 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 estimation 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 13571. The values obtained here are derived from rat lethality data. The FED values from ISO 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 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 specimen in the physical fire model shall be relevant to the appropriate fire exposure of the commercial product or assembly.

6.1.3 Repeatability and interlaboratory 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 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 % by volume to 1 % by volume (0 µl/l to 10 000 µl/l);

— carbon dioxide, 0 % by volume to 10 % by volume (0 µl/l to 100 000 µl/l);

— oxygen, 0 % by volume to 21 % by volume (0 µl/l to 210 000 µl/l).

6.2.3 Other gas analyses (for example, for HCN, HCl, HBr, NO_x, SO_2, 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 and ISO 19702.

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 ensure that they are in compliance with all pertinent regulations regarding release and/or disposal of combustion products or gases.

8 Test specimens

8.1 Test specimens 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 specimens 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 mass 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 O_2, CO_2, 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 during a test. For calibration of the O_2 analyser, ambient air (20,9 % O_2 by volume if the air is dry) shall be used, while for the CO_2 and CO analysers, bottled gases containing CO_2 or CO at known concentration are required. A single mixture containing both CO and CO_2 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 CO_2 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 or ISO 19702.

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

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

11 Calculations

11.1 General

The predicted lethal toxic potency (LC_{50}) of the effluent from the test specimen is calculated from the combustion atmosphere analytical data for CO, CO_2, O_2, 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 m^3.

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 coworkers[3].

11.2.2 Formula (2) was developed empirically by Levin and coworkers (summarized in Reference[4] with citations of the original research) from exposure of laboratory rats to individual and mixed gases.

$$L_{\text{FED}} = \frac{m[\text{CO}]}{[\text{CO}_2]-b} + \frac{21-[\text{O}_2]}{21-\text{LC}_{50,\text{O}_2}} + \frac{[\text{HCN}]}{\text{LC}_{50,\text{HCN}}} + \frac{[\text{HCl}]}{\text{LC}_{50,\text{HCl}}} + \frac{[\text{HBr}]}{\text{LC}_{50,\text{HBr}}} \quad (2)$$

which reduces to

$$L_{\text{FED}} = \frac{m[\text{CO}]}{[\text{CO}_2]-b} + \frac{21-[\text{O}_2]}{(21-5{,}4)} + \frac{[\text{HCN}]}{150} + \frac{[\text{HCl}]}{3\,700} + \frac{[\text{HBr}]}{3\,000}$$

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 µl/l x 10^{-4} (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 microlitres per litre;

$LC_{50,HCN}$ 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 CO_2. If $[CO_2] \leq 5$ % by volume, $m = -18$ and b = 122 000 µl/l. If $[CO_2] > 5$ % by volume, $m = 23$ and b = −38 600 µl/l. Confirmatory work using this model has been published by Pauluhn[5].

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 LC_{50} 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 US National Institute of Standards and Technology (NIST). The “N-Gas Model” takes into account the effects of CO_2 on the toxicity of CO, as expressed empirically from rat exposure studies conducted at NIST. Formula (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[4].

11.2.3 Formula (3) was developed by Purser[6], fitting the rat LC_{50} data obtained mainly by Levin et al.[4] and Kaplan and Hartzell[7].

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

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_{CO2} is a multiplication factor for CO_2-driven hyperventilation, equal to 1 + e[(0,14 · CO_2) −1]/2;

Z_A is an acidosis factor, equal to [CO_2] × 0,05.

The 30-min LC_{50} values used in Formula (3) are given in Table 1.

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

Fire effluent gas	30-min LC_{50} µl/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 Formulae (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 Formula (3).

NOTE 2 There are functional form differences in the way [CO], [CO_2], and depleted O_2 are included in Formulae (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 ± 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 >> 1 are not compatible with survival for times near 30-min, a reasonable estimate of agreement among the two equation calculations is ± 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 Formula (4):

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

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} is expressed in grams per cubic metre.

FED values used in Formula (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 ± 30 % if the concentrations of all the contributing toxicants are measured and included.

However, if toxicants in addition to those included in Formulae (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 the test specimen, 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:

   1) ($C{\cdot}t$) product for each analysed toxicant,

   2) ($C{\cdot}t$) product for each analysed toxicant divided by 30 min,

   3) indication whether Formula (2) or Formula (3) was used,

   4) FED for each test, and

   5) 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 Formula (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 Formulae (2), (3) and (4) is ± 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.

Annex A
(informative)

Optional bioassay for confirmation of predicted LC_{50} values

A.1 General

A predicted LC_{50} 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 LC_{50} 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 judgement 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 LC_{50} values must also comply with good laboratory practice regulations (see References[8] and[9]) to ensure the quality and integrity of data obtained and adhere to applicable regulations with regard to the care and use of experimental animals.

A.2 General guidance

A.2.1 Test animals shall be inbred, healthy, young adult, male or female rats; (see A.3.3 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[8] and the Good Laboratory Practice Regulations[9].

A.2.6 Prior to exposure, the animals shall be weighed and secured into individual head- or nose-only exposure restrainers for placement into the animal exposure chamber.

The animal restraint system must not cause undue physical stress.

A.2.7 Unduly compromising test conditions with regard to oxygen concentration and temperature must not be used.

A.2.8 After testing, surviving animals shall be housed in an animal room separate from the pre-test animal room for at least a 14-day post-exposure observation period.

Any deaths during the post-exposure period shall be recorded.

A.2.9 In tests using exposure of animals, those surviving shall be observed for any signs of toxic effects in accordance with the guidance of Reference[10].

A.3 Procedures

A.3.1 Test procedures conducted for exposure of rats must be comparable to those used to obtain analytical data for the prediction of LC_{50} values; see Clauses 6, 7, 8, 9 and 10 of this International Standard.

A.3.2 As an example of the experimental confirmation of a predicted LC_{50} value, six rats, restrained for head-only exposure, may be exposed to that amount of the test material whose mass loss concentration during the 30-min period is approximately (70 ± 10) % and to (130 ± 10) % of its average predicted LC_{50}.

If no more than one rat dies during the 30-min exposure, or within 14 days post-exposure, to the mass loss concentration corresponding to 70 % of the predicted LC_{50} and at least five rats die during the 30-min exposure, or within 14 days post-exposure, to the mass loss concentration corresponding to 130 % of the LC_{50}, the predicted LC_{50} is considered to be confirmed as an approximate LC_{50}. Alternatively, the rats may be exposed to that concentration of fire effluents representing the predicted LC_{50}, with resultant partial lethality being regarded as confirmation. If confirmation is not successful, or if unexplained or unusual toxicity is suspected, further testing might need to be employed to determine a statistically valid LC_{50} for the test material.

A.3.3 It is the intent of this method that 30-min LC_{50} values for rats be the standard for the reporting of results.

It is also the intent that exposure of rats be used, if necessary, to confirm predicted LC_{50} values. However, exposure of mice may also be used for the confirmation, provided that the FED calculations used employ LC_{50} values for fire gas toxicants that were determined using mice. Once the predicted LC_{50} values for mice are confirmed experimentally, for reporting purposes the analytical data must then be used in FED calculations that are based on rat LC_{50} values. Thus, although mice may be used for confirmation of predicted LC_{50} values, test results are still to be reported as LC_{50} values for rats.

A.3.4 If deemed appropriate in tests using exposure of animals, blood samples may be taken in an acceptable manner immediately after exposure and analysed for carboxyhaemoglobin saturation and cyanide content.

A.4 Calculations

Refer to Clause 11.

A.5 Test report

A.5.1 In addition to data to be reported for the determination of predicted LC_{50} values (Clause 11 of this International Standard), bioassay tests shall provide the following additional information:

a) strain of rat and identity of supplier;

b) mass of each animal when received, prior to test and at 7 and 14 days post-exposure for surviving animals;

c) the number of animals dying during the test (including up to 10 min post-test) and the number of animals that die up to 14 days post-test;

d) blood carboxyhaemoglobin saturation or other blood values if measured;

e) animal observations taken daily during test, for example, unusual behaviour;

f) immediate post-test observations of live animals such as tremors, convulsions, difficulty in breathing, severe eye irritation, etc.

A.5.2 The report shall state whether animal tests did or did not confirm the value of the predicted LC_{50}.

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