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Determination of the lethal toxic potency of fire effluents

Détermination du pouvoir toxique létal des effluents du feu

Reference number
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Foreword

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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.

International Standard ISO 13344 was prepared by Technical Committee ISO/TC 92, *Fire safety*, Subcommittee SC 3, *Toxic hazards in fire*.

Annexes A and B of this International Standard are for information only.

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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.

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Determination of the lethal toxic potency of fire effluents

1 Scope

1.1 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 laboratory fire model. The lethal toxic potency values are specifically related to the fire model selected, the exposure scenario and the material evaluated.

1.2 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) and other toxicants which have been demonstrated to be appropriate. If the fire effluent toxic potency cannot be attributed to the toxicants analyzed, this is an indication that other toxicants or factors must be considered.

1.3 This International Standard is intended to be used to measure and describe the toxic potency of fire effluent atmospheres produced from materials, products or assemblies under controlled laboratory conditions and should not be used 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 which takes into account all of the factors which are pertinent to an assessment of the fire hazard of a particular end use.

1.4 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 standard to establish appropriate safety and health practices.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO/TR 9122-3:1993, *Toxicity testing of fire effluents — Part 3: Methods for the analysis of gases and vapours in fire effluents.*

ISO/TR 9122-4:1993, *Toxicity testing of fire effluents — Part 4: The fire model (furnaces and combustion apparatus used in small-scale testing).*

ISO/TR 9122-5:1993, *Toxicity testing of fire effluents — Part 5: Prediction of toxic effects of fire effluents.*

3 Definitions

For the purposes of this International Standard, the following definitions 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 as a function of time.

3.3 $C \cdot t$ product: Concentration–time product, expressed in parts per million minute (ppm·min) obtained by integration of the area under a concentration–time curve.

3.4 exposure dose: $C \cdot t$ product of a gaseous toxicant available for inhalation.

3.5 fire model: Laboratory combustion device and the conditions under which it is operated.

3.6 fractional effective dose (FED): Ratio of the $C \cdot t$ product for a gaseous toxicant produced in a given test to that $C \cdot t$ product of the toxicant which has been statistically determined from independent experimental data to produce an effect (lethality) in 50 % of test animals within a specified exposure and post-exposure time. Since time values in this ratio mathematically cancel, the FED is also simply the ratio of the average concentration of a gaseous toxicant to its LC_{50} value for the same exposure time. When not used with reference to a specific toxicant, the term FED represents the summation of FEDs for individual toxicants in a combustion atmosphere.

3.7 LC_{50}; lethal concentration 50 %: Concentration of gas or smoke statistically calculated from concentration–response data to produce lethality in 50 % of test animals within a specified exposure and post-exposure time.

NOTE — This is a measure of lethal toxic potency.

3.8 predicted LC_{50}: LC_{50} calculated from combustion atmosphere analytical data according to the method of this International Standard.

3.9 mass charge concentration: Amount of a test specimen placed in a combustion chamber per unit exposure volume or total air flow, expressed in grams per cubic metre.

3.10 mass loss concentration: Amount of a test specimen consumed during combustion per unit exposure volume or total air flow, expressed in grams per cubic metre.

3.11 toxic hazard: Potential for physiological harm from toxic products of combustion.

4 Principle

4.1 This method subjects a test specimen to the combustion conditions of a specific laboratory 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 being determined from integration of the areas under the respective concentration–time plots. The $C \cdot t$ product data, along with either the mass charge or the mass loss of the test specimen during the test, are then used in calculations to predict the 30-min LC_{50} of the test specimen. If considered necessary, the predicted LC_{50} may then be experimentally confirmed as precisely as toxicologically relevant (annex A). Confirmation assures that the monitored toxicants account for the observed toxic effects.

4.2 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. It employs methodology for the calculation of toxic potencies from combustion product analytical

data without the exposure of experimental animals. Such methodology is based on extensive experimentation using exposure of rats to the common fire gases, both singly and in combinations. Expressed mathematically, the principle is shown in equation (1):

$$\text{FED} = \sum_{i=1}^{n} \int_{t_0}^{t} \frac{C_i}{(C \cdot t)_i} \, dt \qquad \ldots (1)$$

where

C_i is the concentration of the toxic component, i, expressed in parts per million (ppm);

$(C \cdot t)_i$ is the concentration–time product, expressed in parts per million minute (ppm·min) of the specific exposure dose 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 toxic fire hazard as a means for the evaluation of materials and products and to assist research and development. The data are not, in themselves, an indication of toxic hazard, or relative toxic hazard, nor are they to be used in the absence of toxic hazard assessment in the regulation of products of commerce.

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 may 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 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 in fire situations can only be inferred to the extent that the rat is correlated with the human as a biological system.

5.6 This test method does not assess incapacitation. Incapacitation may be inferred from lethal toxic potency values.

5.7 This test method does not quantitatively address sensory and upper respiratory tract irritation.

6 Apparatus requirements

6.1 The fire model

6.1.1 The 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 in ISO/TR 9122-4.

6.1.2 Repeatability of results using the fire model shall be demonstrated.

6.1.3 Interlaboratory reproducibility of results using the fire model shall be demonstrated.

6.1.4 The fire model shall be adaptable to animal exposure procedures.

6.1.5 The fire model shall be adaptable to analytical requirements.

6.1.6 The fire model shall be safe to operating personnel.

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 10 000 ppm;

carbon dioxide, 0 to 10 %;

oxygen, 0 to 21 %.

6.2.3 Other gas analyses (for example, HCN, HCl, HBr, NO_x, SO_2, acrolein, formaldehyde and other chemical species) shall be performed, as necessary, by a method of choice with guidance from ISO/TR 9122-3.

7 Hazards

7.1 This test procedure involves combustion processes. Therefore, hazards to operating personnel may 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 must 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 specimens

8.1 Test specimens shall be prepared in accordance with the operating restrictions and conditions applicable to the fire model used.

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 Fire model calibrations

Calibrations shall be conducted in accordance with the applicable operating methodology of the fire model.

9.2 Gas analyser calibrations

9.2.1 At the beginning of each series of tests, the gas analysers (for O_2, CO_2 and CO) shall be calibrated using nitrogen gas for "zeroing" and an appropriate gas mixture near 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 O_2 analyser, ambient air (20,9 % O_2) shall be used, while for the CO_2 and CO analysers bottled gases containing CO_2 and 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 must 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/TR 9122-3.

10 Procedures

10.1 General

These test procedures are designed to produce analytical data for CO, CO_2, O_2, and, if present, HCN, HCl and other toxicants. Choice of specimen size for initial tests is made with consideration of anticipated toxicant yields such that FEDs from 0,5 to 1,5 are obtained (see clause 11). Analytical data from at least two tests are used for calculation of a predicted LC_{50} for the test specimen (clause 12).

10.2 Preparation for tests

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

10.3 Test procedure for obtaining data

10.3.1 Weigh the test specimen and subject it to the operating conditions of the 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.

10.3.3 At the end of 30 min, cease collecting data.

10.3.4 Remove the test specimen from the combustion device and cool it to ambient temperature in an exhaust hood. After the specimen has cooled, determine the mass of the residue.

11 Calculations

11.1 General

The predicted lethal toxic potency (LC_{50}) of 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 specimen mass by first calculating the FED for the test. The LC_{50} is then calculated as that specimen mass which would yield a FED equal to 1 within a volume of 1 m^3.

11.2 Calculation of FED

11.2.1 The 30-min FED for a given specimen mass may be calculated from equation (2):

$$\text{FED} = \frac{[\text{CO}]}{\text{LC}_{50,\,\text{CO}}} + \frac{[\text{HCN}]}{\text{LC}_{50,\,\text{HCN}}} + \frac{[\text{HCl}]}{\text{LC}_{50,\,\text{HCl}}} + \frac{[C_i]}{\text{LC}_{50,\,i}} \quad \ldots (2)$$

where

[CO] is the concentration of CO, in parts per million;

[HCN] is the concentration of HCN, in parts per million;

[HCl] is the concentration of HCl, in parts per million;

$[C_i]$ is the concentration of component i, in parts per million;

$\text{LC}_{50,\,\text{CO}}$ is the LC_{50} for CO, in parts per million;

$\text{LC}_{50,\,\text{HCN}}$ is the LC_{50} for HCN, in parts per million;

$\text{LC}_{50,\,\text{HCl}}$ is the LC_{50} for HCl, in parts per million;

$\text{LC}_{50,\,i}$ is the LC_{50} for component i, in parts per million.

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 which have been statistically determined from independent experimental data to produce lethality in 50 % of test animals (rats) within a 30-min exposure plus 14 days post-exposure.

11.2.2 As an alternative to the use of equation (2), equation (3) may be used to calculate the 30-min FED in cases where oxygen vitiation is significant and CO_2 concentrations are sufficiently high (> 1 %) as to have significant impact on the toxicity of CO.

$$\text{FED} = \frac{m[\text{CO}]}{[\text{CO}_2] - b} + \frac{21 - [\text{O}_2]}{21 - \text{LC}_{50,\,\text{O}_2}} + \frac{[\text{HCN}]}{\text{LC}_{50,\,\text{HCN}}} + \frac{[\text{HCl}]}{\text{LC}_{50,\,\text{HCl}}} + \frac{[\text{HBr}]}{\text{LC}_{50,\,\text{HBr}}} \quad \ldots (3)$$

Therefore

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

where

m and b are respectively the slope and intercept of the interactive curve of CO and CO_2 which depicts the increasing toxicity of CO as CO_2 concentration increases;

$[\text{O}_2]$ is the concentration of O_2, in percent by volume;

[HBr] is the concentration of HBr, in parts per million;

$[\text{CO}_2]$ is the concentration of CO_2, in percent by volume;

$\text{LC}_{50,\,\text{O}_2}$ is the LC_{50} for O_2, in percent by volume;

$\text{LC}_{50,\,\text{HBr}}$ is the LC_{50} for HBr, in parts per million.

The values of all gas concentrations are the integrated $C{\cdot}t$ product values under their respective concentration–time curves taken over the 30-min test period divided by 30. Note that all the values are in ppm except O_2 and CO_2 which are in %. The values of m and b depend on the concentration of CO_2. If $[\text{CO}_2] \leqslant 5$ %, $m = -18$ and $b = 122\,000$. If $[\text{CO}_2] > 5$ %, $m = 23$ and $b = -38\,600$.

NOTE — This use of the FED principle has been termed the "N-Gas Model" by the 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 studies conducted as NIST. Equation (3) also takes into consideration oxygen vitiation, should that be significant. Examination of a series of pure gaseous toxicant experiments in which various percentages of animals 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 0,20.

11.2.3 As an alternative to the use of equation (3), equation (4) takes into account the hyperventilatory effect of CO_2 on all other toxicants using a multiplier, V_{CO_2}, and the direct toxic effects of CO_2 using an additive factor, a.

$$FED = \left(\frac{[CO]}{LC_{50,\,CO}} + \frac{[HCN]}{LC_{50,\,HCN}} + \frac{[AGI]}{LC_{50,\,AGI}} + \frac{[OI]}{LC_{50,\,OI}} \right) \times V_{CO_2} + a \qquad \ldots (4)$$

where

[AGI] is the concentration of acid gas irritants, in parts per million;

[OI] is the concentration of organic irritants, in parts per million;

$LC_{50,\,AGI}$ is the LC_{50} of acid gas irritants, in parts per million;

$LC_{50,\,OI}$ is the LC_{50} of organic irritants, in parts per million;

V_{CO_2} and a are factors, see table 1.

The values of all gas concentrations are the integrated $C \cdot t$ product values under their respective concentration–time curves taken over the 30-min test period, divided by 30. Values of V_{CO_2} and a depend on the concentration of CO_2 in accordance with table 1. The terms involving irritants refer to the summation of acid gas and organic irritant concentrations relative to their LC_{50} values. The oxygen vitiation term of equation (3) may also be used as an additive term if the concentration of oxygen falls below 15 %.

Table 1 — Dependence of V_{CO_2} and a on CO_2 concentration

CO_2 %	V_{CO_2}	a
≤ 2,5	1	0
3,0	1,25	0,1
4,0	1,4	0,2
5,0	1,5	0,25

11.2.4 For each individual toxicant, the LC_{50} values shown in equations (2), (3) and (4) were statistically determined from independent experimental data to produce lethality in 50 % of test animals (rats) within a 30-min exposure plus 14 days post-exposure. Refer to ISO/TR 9122-5 for tabulation of LC_{50} data. Generally accepted 30-min LC_{50} values for exposure of rats to the common fire effluent gases are given in table 2.

Table 2 — 30-min LC_{50} values

Fire effluent gas	30-min LC_{50} ppm
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 — The complete solutions of equations (2), (3) and (4) require that terms be included for all important irritants, such as inorganic acid gases (for example, HCl, HBr, SO_2, NO_x) as well as organic irritants (for example, aldehydes). These terms should be included where appropriate data are available. Organic irritants would be expected to be most significant under conditions for the simulation of nonflaming oxidation and poorly ventilated flaming fires. With these conditions, it may be prudent to conduct animal exposure confirmation of predicted LC_{50} values (see annex A).

11.3 Calculation of predicted LC_{50}

The predicted 30-min LC_{50} for each test specimen in a series of tests is calculated from equation (5):

$$LC_{50} = \frac{m}{FED \times V} \quad \ldots (5)$$

where

m is the specimen mass, in grams, which, according to the guidance of ISO/TR 9122-2, may be either mass charged or mass loss;

V is the total air volume, in cubic metres.

The resulting predicted LC_{50} has the units of grams per cubic metre.

NOTE — FED values used in equation (5) 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.

12 Test report

12.1 The test 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 specimen;

f) 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 specimen mass and mass loss during test in grams per cubic metre of air volume;

j) observations of specimen, including melting, char formation, spalling, unusually vigorous burning and reignition;

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) calculations of

- $C \cdot t$ product for each analysed toxicant,
- $C \cdot t$ product for each analysed toxicant divided by 30 min,
- FED for each test,
- predicted LC_{50}, specifying the calculation method used and the basis for the predicted LC_{50} as either mass charged or mass loss;

m) optionally, plots of individual toxicant concentrations, specimen 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 may be the mean LC_{50} value or may preferably be accomplished from a linear regression analysis of a plot of specimen mass versus FED values. The mass value corresponding to a FED equal to 1 is then used in equation (5) to calculate the best predicted LC_{50} value.

13 Precision and bias

13.1 The precision of this test method has not yet been established.

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 Introduction

A predicted LC_{50} may be experimentally confirmed, assuring that the monitored toxicants account for the observed toxic effects. 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. Every effort must be made to minimize or even avoid the use of such animal exposures, especially in those cases where professional judgement would expect minimal value to be realized.

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 [4] to assure 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 and/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 are not to be used. Weight gain between arrival and 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 should be performed in accordance with applicable recognized guidelines, such as the OECD Guidelines for Testing of Chemicals [5] and Good Laboratory Practice Regulations [4].

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 should not cause undue physical stress.

A.2.7 Unduly compromising test conditions with regard to oxygen concentration and temperature should 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 ISO/TR 9122-2.

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. Refer to 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 (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 may 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 which 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 of this International Standard.

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 seven 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 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}.

Annex B
(informative)

Bibliography

[1] ISO/TR 9122-1:1989, *Toxicity testing of fire effluents — Part 1: General.*

[2] ISO/TR 9122-2:1990, *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).*

[3] ISO/TR 9122-6:1994, *Toxicity testing of fire effluents — Part 6: Guidance for regulators and specifiers on the assessment of toxic hazards in fires in buildings and transport.*

[4] *Good Laboratory Practice Regulations, Toxic Substances Control Act*, 40 CFR Part 792.

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