

Commission Directive 2004/73/EC of 29 April 2004 adapting to technical progress for the twenty-ninth time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances (Text with EEA relevance)

- Article 1 Directive 67/548/EEC is amended as follows: Annex I is amended...
- Article 2 (1) Member States shall bring into force the laws, regulations...
- Article 3 This Directive shall enter into force on the twentieth day...
- Article 4 This Directive is addressed to the Member States.
- Signature

ANNEX 1A

Note K:

ANNEX2A

A.21. OXIDIZING PROPERTIES (LIQUIDS)

1. METHOD
 - 1.1 INTRODUCTION
 - 1.2 DEFINITIONS AND UNITS
 - 1.3 REFERENCE SUBSTANCE
 - 1.4 PRINCIPLE OF THE TEST METHOD
 - 1.5 QUALITY CRITERIA
 - 1.6 DESCRIPTION OF THE METHOD
 - 1.6.1 Preparation
 - 1.6.1.1 Combustible substance
 - 1.6.1.2 Apparatus
 - 1.6.2 Performance of the test
- 2 DATA
 - 2.1 TREATMENT OF RESULTS
- 3 REPORT
 - 3.1 TEST REPORT
 - 3.2 INTERPRETATION OF THE RESULTS
- 4 REFERENCES
 - (1) Recommendations on the Transport of Dangerous Goods, Manual of Tests...

ANNEX 2B

B.1 bis. ACUTE ORAL TOXICITY - FIXED DOSE PROCEDURE

1. METHOD

1.1	INTRODUCTION
1.2	DEFINITIONS
1.3	PRINCIPLE OF THE TEST METHOD
1.4	DESCRIPTION OF THE TEST METHOD
1.4.1	Selection of animal species
1.4.2	Housing and feeding conditions
1.4.3	Preparation of animals
1.4.4	Preparation of doses
1.5	PROCEDURE
1.5.1	Administration of doses
1.5.2	Sighting study
1.5.3	Main study
1.6	OBSERVATIONS
1.6.1	Body weight
1.6.2	Pathology
2	DATA
3	REPORTING
3.1	Test report
4	REFERENCES
(1)	British Toxicology Society Working Party on Toxicity (1984). Special report:...
(2)	Van den Heuvel, M.J., Dayan, A.D. and Shillaker, R.O. (1987)...
(3)	Van den Heuvel, M.J., Clark, D.G., Fielder, R.J., Koundakjian, P.P.,...
(4)	Whitehead, A. and Curnow, R.N. (1992). Statistical evaluation of the...
(5)	Stallard, N. and Whitehead, A. (1995). Reducing numbers in the...
(6)	Stallard, N., Whitehead, A. and Ridgeway, P. (2002). Statistical evaluation...
(7)	OECD (2001). Guidance Document on Acute Oral Toxicity Testing. Environmental...
(8)	OECD (2000). Guidance Document on the Recognition, Assessment and Use...
(9)	OECD (1998). Harmonised Integrated Hazard Classification for Human Health and...
(10)	Lipnick, R.L., Cotruvo, J.A., Hill, R.N., Bruce, R.D., Stitzel, K.A.,...
(11)	Chan P.K and A.W. Hayes (1994) Chapter 16 Acute Toxicity...

ANNEX 1: FLOW CHART FOR THE SIGHTING STUDY

ANNEX 1: FLOW CHART FOR THE SIGHTING STUDY

ANNEX 2: FLOW CHART FOR THE MAIN STUDY

ANNEX 2: FLOW CHART FOR THE MAIN STUDY

ANNEX 3

CRITERIA FOR CLASSIFICATION OF TEST SUBSTANCES WITH EXPECTED LD50 VALUES...

TESTING AT DOSES ABOVE 2000 MG/KG

Sighting Study

Main Study

ANNEX TEST METHOD B.1 bis - Guidance on classification according to...

ANNEX~~EST~~ METHOD B. 1 bis - Guidance on classification according...

ANNEX2C

B.1 tris. ACUTE ORAL TOXICITY - ACUTE TOXIC CLASS METHOD

1. METHOD
 - 1.1 INTRODUCTION
 - 1.2 DEFINITIONS
 - 1.3 PRINCIPLE OF THE TEST
 - 1.4 DESCRIPTION OF THE METHOD
 - 1.4.1 Selection of animal species
 - 1.4.2 Housing and feeding conditions
 - 1.4.3 Preparation of animals
 - 1.4.4 Preparation of doses
 - 1.5 PROCEDURE
 - 1.5.1 Administration of doses
 - 1.5.2 Number of animals and dose levels
 - 1.5.3 Limit test
 - 1.6 OBSERVATIONS
 - 1.6.1 Body weight
 - 1.6.2 Pathology
2. DATA
3. REPORTING
 - 3.1 Test report
4. REFERENCES
 - (1) Roll R., Höfer-Bosse Th. And Kayser D. (1986). New Perspectives...
 - (2) Roll R., Riebschläger M., Mischke U. and Kayser D. (1989)....
 - (3) Diener W., Sichha L., Mischke U., Kayser D. and Schlede...
 - (4) Diener W., Mischke U., Kayser D. and Schlede E. (1995)....
 - (5) Diener W., and Schlede E. (1999) Acute Toxicity Class Methods:...
 - (6) Schlede E., Mischke U., Roll R. and Kayser D. (1992)....
 - (7) Schlede E., Mischke U., Diener W. and Kayser D. (1994)....
 - (8) OECD (2001) Guidance Document on Acute Oral Toxicity Testing. Environmental...
 - (9) OECD (2000) Guidance Document on the Recognition, Assessment and Use...
 - (10) OECD (1998) Harmonized Integrated Hazard Classification System For Human Health...
 - (11) Lipnick R L, Cotruvo., J A, Hill R N, Bruce...
 - (12) Chan P.K. and A.W. Hayes. (1994). Chap. 16. Acute...

ANNEX~~EST~~ PROCEDURE TO BE FOLLOWED FOR EACH OF THE STARTING DOSES...
GENERAL REMARKS

ANNEX~~EST~~ PROCEDURE WITH A STARTING DOSE OF 5 MG/KG BODY...

ANNEX~~EST~~ PROCEDURE WITH A STARTING DOSE OF 50 MG/KG BODY...

ANNEX~~EST~~ PROCEDURE WITH A STARTING DOSE OF 300 MG/KG BODY...

ANNEX~~EST~~ PROCEDURE WITH A STARTING DOSE OF 2000 MG/KG BODY...

ANNEX 2

CRITERIA FOR CLASSIFICATION OF TEST SUBSTANCES WITH EXPECTED LD50 VALUES...
TESTING AT DOSES ABOVE 2000 MG/KG

ANNEX TEST METHOD B.1 this: Guidance on classification according to EU...

ANNEX TEST METHOD B.1 tris: Guidance on classification according to EU...

ANNEX TEST METHOD B.2 tris: Guidance on classification according to EU...

ANNEX TEST METHOD B.3 tris: Guidance on classification according to EU...

ANNEX 2D

B. 4. ACUTE TOXICITY: DERMAL IRRITATION/CORROSION

1. METHOD

1.1 INTRODUCTION

1.2 DEFINITIONS

1.3 PRINCIPLE OF THE TEST METHOD

1.4 DESCRIPTION OF THE TEST METHOD

1.4.1 Preparation for the in vivo test

1.4.1.1 Selection of animal species

1.4.1.2 Preparation of the animals

1.4.1.3 Housing and feeding conditions

1.4.2 Test procedure

1.4.2.1 Application of the test substance

1.4.2.2 Dose level

1.4.2.3 Initial test (In vivo dermal irritation/corrosion test using one animal)...

1.4.2.4 Confirmatory test (In vivo dermal irritation test with additional animals)...

1.4.2.5 Observation period

1.4.2.6 Clinical observations and grading of skin reactions

2. DATA

2.1 PRESENTATION OF RESULTS

2.2 EVALUATION OF RESULTS

3. REPORTING

3.1 TEST REPORT

4. REFERENCES

(1) Barratt, M.D., Castell, J.V., Chamberlain, M., Combes, R.D., Dearden, J.C.,...

(2) Young, J.R., How, M.J., Walker, A.P., Worth W.M.H. (1988) Classification...

(3) Worth, A.P., Fentem, J.H., Balls, M., Botham, P.A., Curren, R.D.,...

(4) ECETOC (1990) Monograph No. 15, "Skin Irritation", European Chemical Industry,...

(5) Fentem, J.H., Archer, G.E.B., Balls, M., Botham, P.A., Curren, R.D.,...

(5a) Testing Method B.40 Skin Corrosion.

(6) OECD (1996) OECD Test Guidelines Programme: Final Report of the...

- (7) OECD (1998) Harmonized Integrated Hazard Classification System for Human Health...
- (8) OECD (2000). Guidance Document on the Recognition, Assessment and Use...
- (9) EPA (1990). Atlas of Dermal Lesions, (20T-2004). United States Environmental...

TABLE I: GRADING OF SKIN REACTIONS

ANNEXA Sequential Testing Strategy for Dermal Irritation and Corrosion

GENERAL CONSIDERATIONS

DESCRIPTION OF THE EVALUATION AND TESTING STRATEGY

REFERENCES

- (1) OECD (1996). Test Guidelines Programme: Final Report on the OECD...
- (2) OECD (1998). Harmonized Integrated Hazard Classification System for Human Health...
- (3) Worth, A.P., Fentem J.H., Balls M., Botham P.A., Curren R.D.,...
- (4) Young, J.R., How, M.J., Walker, A.P., Worth, W.M.H. (1988). Classification...
- (5) Patil, S.M., Patrick, E., Maibach, H.I. (1996) Animal, Human, and...
- (6) Testing Method B.40.
- (7) Fentem, J.H., Archer, G.E.B., Balls, M., Botham, P.A., Curren, R.D.,...

FIGURE TESTING AND EVALUATION STRATEGY FOR DERMAL IRRITATION / CORROSION

ANNEX 2E

B. 5. ACUTE TOXICITY: EYE IRRITATION/CORROSION

1. METHOD

1.1 INTRODUCTION

1.2 DEFINITIONS

1.3 PRINCIPLE OF THE TEST METHOD

1.4 DESCRIPTION OF THE TEST METHOD

1.4.1 Preparation for the in vivo test

1.4.1.1 Selection of species

1.4.1.2 Preparation of animals

1.4.1.3 Housing and feeding conditions

1.4.2 Test procedure

1.4.2.1 Application of the test substance

1.4.2.2 Irrigation

1.4.2.3 Dose level

1.4.2.4 Initial test (In vivo eye irritation/corrosion test using one animal)...

1.4.2.5 Local anaesthetics

1.4.2.6 Confirmatory test (In vivo eye irritation test with additional animals)...

1.4.2.7 Observation period

2. DATA

2.2 EVALUATION OF RESULTS

3. REPORTING
 - 3.1 TEST REPORT
 - 3.2 INTERPRETATION OF THE RESULTS
4. REFERENCES
 - (1) Barratt, M.D., Castell, J.V., Chamberlain, M., Combes, R.D., Dearden, J.C.,...
 - (2) de Silva, O., Cottin, M., Dami, N., Roguet, R., Catroux,...
 - (3) Worth A.P. and Fentem J.H. (1999) A general approach for...
 - (4) Young, J.R., How, M.J., Walker, A.P., Worth W.M.H. (1988) Classification...
 - (5) Neun, D.J. (1993) Effects of Alkalinity on the Eye Irritation...
 - (6) Fentem, J.H., Archer, G.E.B., Balls, M., Botham, P.A., Curren, R.D.,...
 - (6a) Testing Method B.40 Skin Corrosion.
 - (7) Testing method B.4. Acute toxicity: dermal irritation/corrosion.
 - (8) OECD (1996) OECD Test Guidelines Programme: Final Report of the...
 - (9) OECD (1998) Harmonized Integrated Hazard Classification System for Human Health...
 - (10) OECD (2000) Guidance Document on the Recognition, Assessment and Use...

TABLE I: GRADING OF OCULAR LESIONS

ANNEXA Sequential Testing Strategy for Eye Irritation and Corrosion

GENERAL CONSIDERATIONS

DESCRIPTION OF THE STEPWISE TESTING STRATEGY

REFERENCES

- (1) OECD (1996) OECD Test Guidelines Programme: Final Report of the...
- (2) OECD (1998) Harmonized Integrated Hazard Classification System for Human Health...
- (3) Worth, A.P. and Fentem J.H. (1999). A General Approach for...
- (4) Testing method B.4. Acute Toxicity: dermal irritation/corrosion.
- (5) Young, J.R., How, M.J., Walker, A.P., Worth W.M.H. (1988) Classification...
- (6) Neun, D.J. (1993) Effects of Alkalinity on the Eye Irritation...
- (7) Fentem, J.H., Archer, G.E.B., Balls, M., Botham, P.A., Curren, R.D.,...
- (8) Testing Method B.40 Skin Corrosion.
- (9) Annex to Testing method B.4: A Sequential Testing Strategy for...

FIGURE TESTING AND EVALUATION STRATEGY FOR EYE IRRITATION/CORROSION

ANNEX2F

B.31. PRENATAL DEVELOPMENTAL TOXICITY STUDY

1. METHOD

- 1.1 INTRODUCTION
- 1.2 DEFINITIONS
- 1.3 REFERENCE SUBSTANCE
- 1.4 PRINCIPLE OF THE TEST METHOD
- 1.5 DESCRIPTION OF THE TEST METHOD

	1.5.1	Selection of animal species
	1.5.2	Housing and feeding conditions
	1.5.3	Preparation of the animals
1.6		PROCEDURE
	1.6.1	Number and sex of animals
	1.6.2	Preparation of doses
	1.6.3	Dosage
	1.6.4	Limit test
	1.6.5	Administration of doses
	1.6.6	Observations of the dams
	1.6.7	Body weight and food consumption
	1.6.8	Post-mortem examination
	1.6.9	Examination of uterine contents
	1.6.10	Examination of foetuses
2		DATA
	2.1	TREATMENT OF RESULTS
	2.2	EVALUATION OF RESULTS
	2.3	INTERPRETATION OF RESULTS
3		REPORTING
		TEST REPORT
4		REFERENCES
	(1)	Kavlock R.J. et al. (1996) A Simulation Study of the...
	(2)	Kimmel, C.A. and Francis, E.Z. (1990) Proceedings of the Workshop...
	(3)	Wong, B.A., et al. (1997) Developing Specialized Inhalation Exposure Systems...
	(4)	US Environmental Protection Agency (1985) Subpart E-Specific Organ/Tissue Toxicity, 40...
	(5)	Salewski, E. (1964) Faerbermethode zum Makroskopischen Nachweis von Implantations Stellen...
	(6)	Edwards, J.A. (1968) The external Development of the Rabbit and...
	(7)	Inouye, M. (1976) Differential Staining of Cartilage and Bone in...
	(8)	Igarashi, E. et al. (1992) Frequency Of Spontaneous Axial Skeletal...
	(9)	Kimmel, C.A. et al. (1993) Skeletal Development Following Heat Exposure...
	(10)	Marr, M.C. et al. (1988) Comparison of Single and Double...
	(11)	Barrow, M.V. and Taylor, W.J. (1969) A Rapid Method for...
	(12)	Fritz, H. (1974) Prenatal Ossification in Rabbits ss Indicative of...
	(13)	Gibson, J.P. et al. (1966) Use of the Rabbit in...
	(14)	Kimmel, C.A. and Wilson, J.G. (1973) Skeletal Deviation in Rats:...
	(15)	Marr, M.C. et al. (1992) Developmental Stages of the CD...
	(16)	Monie, I.W. et al. (1965) Dissection Procedures for Rat Foetuses...
	(17)	Spark, C. and Dawson, A.B. (1928) The Order and Time...
	(18)	Staples, R.E. and Schnell, V.L. (1964) Refinements in Rapid Clearing...
	(19)	Strong, R.M. (1928) The Order Time and Rate of Ossification...
	(20)	Stuckhardt, J.L. and Poppe, S.M. (1984) Fresh Visceral Examination of...
	(21)	Walker, D.G. and Wirtschafter, Z.T. (1957) The Genesis of the...
	(22)	Wilson, J.G. (1965) Embryological Considerations in Teratology. In Teratology: Principles...
	(23)	Wilson, J.G. and Fraser, F.C. (eds). (1977) Handbook of Teratology,...
	(24)	Varnagy, L. (1980) Use of Recent Fetal Bone Staining Techniques...
	(25)	Staples, R.E. (1974) Detection of visceral Alterations in Mammalian Foetuses....

- (26) Van Julsingha, E.B. and C.G. Bennett (1977) A Dissecting Procedure...
- (27) US Environmental Protection Agency (1991) Guidelines for Developmental Toxicity Risk...
- (28) Wise, D.L. et al. (1997) Terminology of Developmental Abnormalities in...

ANNEX2G

B.35. TWO-GENERATION REPRODUCTION TOXICITY STUDY

1. METHOD

1.1 INTRODUCTION

1.2 PRINCIPLE OF THE TEST METHOD

1.3 DESCRIPTION OF THE TEST METHOD

1.3.1 Selection of animal species

1.3.2 Housing and feeding conditions

1.3.3 Preparation of animals

1.4 PROCEDURE

1.4.1 Number and sex of animals

1.4.2 Preparation of Doses

1.4.3 Dosage

1.4.4 Limit test

1.4.5 Administration of doses

1.4.6 Experimental schedules

1.4.7 Mating procedure

1.4.7.1 Parental (P) mating

1.4.7.2 F1 mating

1.4.7.3 Second mating

1.4.7.4 Litter size

1.5 OBSERVATIONS

1.5.1 Clinical observations

1.5.2 Body weight and food/water consumption of parent animals

1.5.3 Oestrus cycle

1.5.4 Sperm parameters

1.5.5 Offspring

1.5.6 Gross necropsy

1.5.7 Organ weights

1.5.8 Histopathology

1.5.8.1 Parental Animals

1.5.8.2 Weanlings

2 DATA

2.1 TREATMENT OF RESULTS

2.2 EVALUATION OF RESULTS

2.3 INTERPRETATION OF RESULTS

3 REPORTING

TEST REPORT

4 REFERENCES

- (1) Sadleir, R.M.F.S. (1979). Cycles and Seasons, In: Reproduction in Mammals:...
- (2) Gray, L.E. et al., (1989). A Dose-Response Analysis of Methoxychlor-Induced...
- (3) Robb, G.W. et al., (1978). Daily Sperm Production and Epididymal...

- (4) Klinefelter, G.R. et al., (1991). The Method of Sperm Collection...
- (5) Seed, J. et al. (1996). Methods for Assessing Sperm Motility,...
- (6) Chapin, R.E. et al., (1992). Methods for Assessing Rat Sperm Motility...
- (7) Klinefelter, G.R. et al., (1992). Direct Effects of Ethane Dimethanesulphonate...
- (8) Slott, V.L. et al., (1991). Rat Sperm Motility Analysis: Methodologic...
- (9) Slott, V.L. and Perreault, S.D., (1993). Computer-Assisted Sperm Analysis of...
- (10) Toth, G.P. et al. (1989). The Automated Analysis of Rat...
- (11) Working, P.K. and M. Hurtt, (1987). Computerized Videomicrographic Analysis of...
- (12) Linder, R.E. et al., (1992). Endpoints of Spermatotoxicity in the...
- (13) Korenbrot, C.C. et al., (1977). Preputial Separation as an External...
- (14) Russell, L.D. et al., (1990). Histological and Histopathological Evaluation of...
- (15) Heindel, J.J. and R.E. Chapin, (eds.) (1993). Part B. Female...
- (16) Heindel, J.J. et al., (1989) Histological Assessment of Ovarian Follicle...
- (17) Manson, J.M. and Y.J. Kang, (1989). Test Methods for Assessing...
- (18) Smith, B.J. et al., (1991). Comparison of Random and Serial...
- (19) Heindel, J.J. (1999). Oocyte Quantitation and Ovarian Histology. In: An...
- (20) Thomas, J. A. (1991). Toxic Responses of the Reproductive System....
- (21) Zenick, H. and E.D. Clegg, (1989). Assessment of Male Reproductive...
- (22) Palmer, A.K. (1981). In: Developmental Toxicology, Kimmel, C.A. and J....
- (23) Palmer, A.K. (1978). In Handbook of Teratology, Vol. 4, J.G....

ANNEX 2H

B.42. SKIN SENSITISATION: LOCAL LYMPH NODE ASSAY

1. METHOD

1.1 INTRODUCTION

1.2 PRINCIPLE OF THE TEST METHOD

1.3 DESCRIPTION OF THE TEST METHOD

1.3.1 Preparations

1.3.1.1 Housing and feeding conditions

1.3.1.2 Preparation of animals

1.3.2 Test Conditions

1.3.2.1 Experimental animals

1.3.2.2 Reliability check

1.3.2.3 Number of animals, dose levels and vehicle selection.

1.3.3 Test procedure

1.3.3.1 Experimental schedule

1.3.3.2 Preparation of cell suspensions

1.3.3.3 Determination of cell proliferation (incorporated radioactivity)

1.3.3.4 Observations

1.3.4 Calculation of results

- 2 DATA
- 3 REPORTING
 - TEST REPORT
- 4 REFERENCES
 - 1 Kimber, I. and Basketter, D.A. (1992). The murine local lymph...
 - 2 Kimber, I, Derman, R.J., Scholes E.W, and Basketter, D.A. (1994)....
 - 3 Kimber, I., Hilton, J., Dearman, R.J., Gerberick, G.F., Ryan, C.A.,...
 - 4 Testing Method B.6.
 - 5 Chamberlain, M. and Basketter, D.A. (1996). The local lymph node...
 - 6 Basketter, D.A., Gerberick, G.F., Kimber, I. and Loveless, S.E (1996)....
 - 7. Basketter, D.A., Gerberick, G.F. and Kimber, I. (1998). Strategies for...
 - 8 Van Och, F.M.M, Slob, W., De Jong, W.H., Vandebriel, R.J.,...
 - 9 Dearman, R.J., Hilton, J., Evans, P., Harvey, P., Basketter, D.A....
 - 10 National Institute of Environmental Health Sciences (1999). The Murine Local...
 - 11 Testing method B.4.
 - 12 Basketter, D.A., Selbie, E., Scholes, E.W. Lees, D. Kimber, I....
 - 13 Basketter D.A., Lea L.J., Dickens A., Briggs D., Pate I.,...
 - 14 Basketter DA, Blaikie L, Derman RJ, Kimber I, Ryan CA,...

B.43. NEUROTOXICITY STUDY IN RODENTS

- 1. METHOD
 - 1.1 INTRODUCTION
 - 1.2 DEFINITIONS
 - 1.3 PRINCIPLE OF THE TEST METHOD
 - 1.4 DESCRIPTION OF THE TEST METHOD
 - 1.4.1 Selection of animal species
 - 1.4.2 Housing and feeding conditions
 - 1.4.3 Preparation of animals
 - 1.4.4 Route of administration and preparation of doses
 - 1.5 PROCEDURES
 - 1.5.1 Number and sex animals
 - 1.5.2 Treatment and control group
 - 1.5.3 Reliability check
 - 1.5.4 Dose selection
 - 1.5.5 Limit test
 - 1.5.6 Administration of doses
 - 1.6 OBSERVATION
 - 1.6.1 Frequency of observations and tests
 - 1.6.1.1 Observations of general health condition and mortality/morbidity
 - 1.6.1.2 Detailed clinical observations
 - 1.6.1.3 Functional tests
 - 1.6.2 Body weight and food/water consumption
 - 1.6.3 Ophthalmology
 - 1.6.4 Haematology and clinical biochemistry
 - 1.6.5 Histopathology
- 2 DATA
 - 2.1 TREATMENT OF RESULTS
 - 2.2 EVALUATION AND INTERPRETATION OF RESULTS
- 3 REPORTING
- 4 REFERENCES

1. OECD Guidance Document on Neurotoxicity Testing Strategies and Test Methods....
2. Test Guideline for a Developmental Neurotoxicity Study, OECD Guidelines for...
3. World Health Organization (WHO) (1986). Environmental Health Criteria document 60:...
4. Spencer, P.S. and Schaumburg, H.H. (1980). Experimental and Clinical Neurotoxicology....
5. Tupper, D.E. and Wallace, R.B. (1980). Utility of the Neurological...
6. Gad, S.C. (1982). A Neuromuscular Screen for Use in Industrial...
7. Moser, V.C., McDaniel, K.M. and Phillips, P.M. (1991). Rat Strain...
8. Meyer, O.A., Tilson, H.A., Byrd, W.C. and Riley, M.T. (1979)....
9. Crofton, K.M., Haward, J.L., Moser, V.C., Gill, M.W., Reirer, L.W.,...
10. Tilson, H.A., and Mitchell, C.L. eds. (1992). Neurotoxicology Target Organ...
11. Chang, L.W., ed. (1995). Principles of Neurotoxicology. Marcel Dekker, New...
12. Broxup, B. (1991). Neuopathology as a screen for Neurotoxicity Assessment....
13. Moser, V.C., Anthony, D.C., Sette, W.F. and MacPhail, R.C. (1992)....
14. O'Callaghan, J.P. (1988). Neurotypic and Gliotypic Proteins as Biochemical Markers...
15. O'Callaghan J.P. and Miller, D.B. (1988). Acute Exposure of the...
16. Fox. D.A., Lowndes, H.E. and Birkamper, G.G. (1982). Electrophysiological Techniques...
17. Johnson, B.L. (1980). Electrophysiological Methods in neurotoxicity Testing. In: Experimental...
18. Bancroft, J.D. and Steven A. (1990). Theory and Praticice of...

ANNEX 2I

C.21. SOIL MICROORGANISMS: NITROGEN TRANSFORMATION TEST

1. METHOD
 - 1.1 INTRODUCTION
 - 1.2 DEFINITIONS
 - 1.3 REFERENCE SUBSTANCES
 - 1.4 PRINCIPLE OF THE TEST METHOD
 - 1.5 VALIDITY OF THE TEST
 - 1.6 DESCRIPTION OF THE TEST METHOD
 - 1.6.1 Apparatus
 - 1.6.2 Selection and number of soils
 - 1.6.3 Collection and storage of soil samples
 - 1.6.3.1 Collection
 - 1.6.3.2 Storage
 - 1.6.4 Handling and preparation of soil for the test
 - 1.6.4.1 Pre-incubation
 - 1.6.4.2 Physical-chemical characteristics
 - 1.6.4.3 Amendment with organic substrate
 - 1.6.5 Preparation of the test substance for the application to soil...
 - 1.6.6 Test concentrations
 - 1.7 PERFORMANCE OF THE TEST

	1.7.1	Conditions of exposure
	1.7.1.1	Treatment and control
	1.7.1.2	Incubation of soil samples
	1.7.1.3	Test conditions and duration
	1.7.2	Sampling and analysis of soils
	1.7.2.1	Soil sampling schedule
	1.7.2.2	Analysis of soil samples
2		DATA
	2.1	TREATMENT OF RESULTS
	2.2	INTERPRETATION OF RESULTS
3		REPORTING
4		REFERENCES
	(1)	EPPO (1994). Decision-Making Scheme for the Environmental Risk Assessment of...
	(2)	BBA (1990). Effects on the Activity of the Soil Microflora...
	(3)	EPA (1987). Soil Microbial Community Toxicity Test. EPA 40 CFR...
	(4)	SETAC-Europe (1995). Procedures for assessing the environmental fate and ecotoxicity...
	(5)	ISO/DIS 14238 (1995). Soil Quality - Determination of Nitrogen Mineralisation...
	(6)	OECD (1995). Final Report of the OECD Workshop on Selection...
	(7)	ISO 10381-6 (1993). Soil quality - Sampling. Guidance on the...
	(8)	ISO 14240-1 (1997). Soil quality - Determination of soil microbial...
	(9)	ISO 14240-2 (1997). Soil quality - Determination of soil microbial...
	(10)	Litchfield, J.T. and Wilcoxon F. (1949). A simplified method of...
	(11)	Finney, D.J. (1971). Probit Analysis. 3rd ed., Cambridge, London and...
	(12)	Finney, D.J. (1978). Statistical Methods in biological Assay. Griffin, Weycombe,...
C.22.		SOIL MICROORGANISMS: CARBON TRANSFORMATION TEST
	1.	METHOD
	1.1	INTRODUCTION
	1.2	DEFINITIONS
	1.3	REFERENCE SUBSTANCES
	1.4	PRINCIPLE OF THE TEST METHOD
	1.5	VALIDITY OF THE TEST
	1.6	DESCRIPTION OF THE TEST METHOD
	1.6.1	Apparatus
	1.6.2	Selection and number of soils
	1.6.3	Collection and storage of soil samples
	1.6.3.1	Collection
	1.6.3.2	Storage
	1.6.4	Handling and preparation of soil for the test
	1.6.4.1	Pre-incubation
	1.6.4.2	Physical-chemical characteristics
	1.6.5	Preparation of the test substance for the application to soil...
	1.6.6	Test concentrations
	1.7	PERFORMANCE OF THE TEST
	1.7.1	Conditions of exposure
	1.7.1.1	Treatment and control
	1.7.1.2	Incubation of soil samples
	1.7.1.3	Test conditions and duration

- 1.7.2 Sampling and analysis of soils
 - 1.7.2.1 Soil sampling schedule
 - 1.7.2.2 Measurement of glucose-induced respiration rates
- 2 DATA
- 2.1 TREATMENT OF RESULTS
- 2.2 INTERPRETATION OF RESULTS
- 3 REPORTING
- TEST REPORT
- 4 REFERENCES
 - (1) EPPO (1994). Decision-Making Scheme for the Environmental Risk Assessment of...
 - (2) BBA (1990). Effects on the Activity of the Soil Microflora....
 - (3) EPA (1987). Soil Microbial Community Toxicity Test. EPA 40 CFR...
 - (4) SETAC-Europe (1995). Procedures for assessing the environmental fate and ecotoxicity...
 - (5) OECD (1995). Final Report of the OECD Workshop on Selection...
 - (6) ISO 10381-6 (1993). Soil quality - Sampling. Guidance on the...
 - (7) Anderson, J.P.E. (1987). Handling and Storage of Soils for Pesticide...
 - (8) Anderson, J.P.E. (1982). Soil Respiration, in "Methods of Soil Analysis...
 - (9) ISO 11266-1. (1993). Soil Quality - Guidance on Laboratory Tests...
 - (10) ISO 14239 (1997E). Soil Quality - Laboratory incubation systems for...
 - (11) Heinemeyer, O., Insam, H., Kaiser, E.A, and Walenzik, G. (1989)....
 - (12) ISO 14240-1 (1997). Soil quality - Determination of soil microbial...
 - (13) ISO 14240-2 (1997). Soil quality - Determination of soil microbial...
 - (14) Malkomes, H.-P. (1986). Einfluß von Glukosemenge auf die Reaktion der...
 - (15) Litchfield, J.T. and Wilcoxon, F. (1949). A simplified method of...
 - (16) Finney, D.J. (1971). Probit Analysis. 3rd ed., Cambridge, London and...
 - (17) Finney D.J. (1978). Statistical Methods in biological Assay. Griffin, Weycombe,...
- C.23. AEROBIC AND ANAEROBIC TRANSFORMATION IN SOIL
 - 1. METHOD
 - 1.1 INTRODUCTION
 - 1.2 DEFINITIONS
 - 1.3 REFERENCE SUBSTANCES
 - 1.4 APPLICABILITY OF THE TEST
 - 1.5 INFORMATION ON THE TEST SUBSTANCE
 - 1.6 PRINCIPLE OF THE TEST METHOD
 - 1.7 QUALITY CRITERIA
 - 1.7.1 Recovery
 - 1.7.2 Repeatability and sensitivity of analytical method
 - 1.7.3 Accuracy of transformation data
 - 1.8 DESCRIPTION OF THE TEST METHOD
 - 1.8.1 Equipment and chemical reagents
 - 1.8.2 Test substance application
 - 1.8.3 Soils
 - 1.8.3.1 Soil selection
 - 1.8.3.2 Collection, handling, and storage of soils
 - 1.9 PERFORMANCE OF THE TEST
 - 1.9.1 Test conditions

- 1.9.1.1 Test temperature
 - 1.9.1.2 Moisture content
 - 1.9.1.3 Aerobic incubation conditions
 - 1.9.1.4 Sterile aerobic conditions
 - 1.9.1.5 Anaerobic incubation conditions
 - 1.9.1.6 Paddy incubation conditions
 - 1.9.1.7 Test duration
 - 1.9.2 Performance of the test
 - 1.9.3 Sampling and measurement
 - 1.9.4 Optional tests
- 2 DATA
- 2.1 TREATMENT OF RESULTS
 - 2.2 EVALUATION AND INTERPRETATION OF RESULTS
- 3 REPORTING
- TEST REPORT
- 4 REFERENCES
- (1) US- Environmental Protection Agency (1982). Pesticide Assessment Guidelines, Subdivision N....
 - (2) Agriculture Canada (1987). Environmental Chemistry and Fate. Guidelines for registration...
 - (3) European Union (EU) (1995). Commission Directive 95/36/EC of 14 July...
 - (4) Dutch Commission for Registration of Pesticides (1995). Application for registration...
 - (5) BBA (1986). Richtlinie für die amtliche Prüfung von Pflanzenschutzmitteln, Teil...
 - (6) ISO/DIS 11266-1 (1994). Soil Quality -Guidance on laboratory tests for...
 - (7) ISO 14239 (1997). Soil Quality - Laboratory incubation systems for...
 - (8) SETAC (1995). Procedures for Assessing the Environmental Fate and Ecotoxicity...
 - (9) MAFF - Japan 2000 - Draft Guidelines for transformation studies...
 - (10) OECD (1995). Final Report of the OECD Workshop on Selection...
 - (11) Guth, J.A. (1980). The study of transformations. In Interactions between...
 - (12) DFG: Pesticide Bound Residues in Soil. Wiley - VCH (1998)....
 - (13) T.R. Roberts: Non-extractable pesticide residue in soils and plants. Pure...
 - (14) OECD Test Guideline 304 A: Inherent Biodegradability in Soil (adopted...
 - (15) ISO 10381-6 (1993). Soil Quality - Sampling - Part 6:...
 - (16) Annex V to Dir. 67/548/EEC
 - (17) Guth, J.A. (1981). Experimental approaches to studying the fate of...
 - (18) Soil Texture Classification (US and FAO systems): Weed Science, 33,...
 - (19) Methods of Soil Analysis (1986). Part 1, Physical and Mineralogical...
 - (20) Methods of Soil Analysis (1982). Part 2, Chemical and Microbiological...
 - (21) ISO Standard Compendium Environment (1994). Soil Quality - General aspects;...
 - (22) Mückenhausen, E. (1975). Die Bodenkunde und ihre geologischen, geomorphologischen, mineralogischen...

- (23) Scheffer, F., Schachtschabel, P. (1975). Lehrbuch der Bodenkunde. F. Enke...
- (24) Anderson, J.P.E., Domsch, K.H. (1978) A physiological method for the...
- (25) ISO 14240-1 and 2 (1997). Soil Quality - Determination of...
- (26) Anderson, J.P.E. (1987). Handling and storage of soils for pesticide...
- (27) Kato, Yasuhiro. (1998). Mechanism of pesticide transformation in the environment:...
- (28) Keuken O., Anderson J.P.E. (1996). Influence of storage on biochemical...
- (29) Stenberg B., Johansson M., Pell M., Sjö Dahl-Svensson K., Stenström J.,...
- (30) Gennari, M., Negre, M., Ambrosoli, R. (1987). Effects of ethylene...
- (31) Anderson, J.P.E. (1975). Einfluss von Temperatur und Feuchte auf Verdampfung,...
- (32) Hamaker, J.W. (1976). The application of mathematical modelling to the...
- (33) Goring, C.A.I., Laskowski, D.A., Hamaker, J.W., Meikle, R.W. (1975). Principles...
- (34) Timme, G., Frehse, H., Laska, V. (1986). Statistical interpretation and...
- (35) Timme, G., Frehse, H. (1980). Statistical interpretation and graphic representation...
- (36) Gustafson D.I., Holden L.R. (1990). Non-linear pesticide dissipation in soil;...
- (37) Hurle K., Walker A. (1980). Persistence and its prediction. In...

ANNEX WATER TENSION, FIELD CAPACITY (FC) AND WATER HOLDING CAPACITY (WHC)(1)...

- (1) Mückenhausen, E. (1975). Die Bodenkunde und ihre geologischen, geomorphologischen, mineralogischen...

ANNEX SOIL MOISTURE CONTENTS (g water per 100 g dry soil)...

ANNEX 3

- (1) Guth, J.A. (1980). The study of transformations. In Interactions between...
- (2) Guth, J.A. (1981). Experimental approaches to studying the fate of...
- (3) Anderson, J.P.E. (1975). Einfluss von Temperatur und Feuchte auf Verdampfung,...

C.24. AEROBIC AND ANAEROBIC TRANSFORMATION IN AQUATIC SEDIMENT SYSTEMS

1. METHOD

- 1.1 INTRODUCTION
- 1.2 DEFINITIONS
- 1.3 REFERENCE SUBSTANCES
- 1.4 INFORMATION ON THE TEST SUBSTANCE
- 1.5 PRINCIPLE OF THE TEST METHOD
- 1.6 APPLICABILITY OF THE TEST
- 1.7 QUALITY CRITERIA
 - 1.7.1 Recovery
 - 1.7.2 Repeatability and sensitivity of analytical method
 - 1.7.3 Accuracy of transformation data
- 1.8 DESCRIPTION OF THE METHOD

- 1.8.1 Test system and apparatus
- 1.8.2 Selection and number of aquatic sediments
 - 1.8.2.1 Sediment selection
 - 1.8.2.2 Characterisation of water-sediment samples
- 1.8.3 Collection, Handling and Storage
 - 1.8.3.1 Collection
 - 1.8.3.2 Handling
 - 1.8.3.3 Storage
- 1.8.4 Preparation of the sediment/water samples for the test
- 1.9 PERFORMANCE OF THE TEST
 - 1.9.1 Test conditions
 - 1.9.2 Treatment and application of test substance
 - 1.9.3 Test duration and sampling
 - 1.9.4 Optional preliminary test
 - 1.9.5 Measurements and analysis
- 2 DATA
- 2.1 TREATMENT OF RESULTS
- 3 REPORTING
- 3.1 TEST REPORT
- 4 REFERENCES
 - (1) BBA-Guidelines for the examination of plant protectors in the registration...
 - (2) Commission for registration of pesticides: Application for registration of a...
 - (3) MAFF Pesticides Safety Directorate. (1992). Preliminary guideline for the conduct...
 - (4) Agriculture Canada: Environmental chemistry and fate. (1987). Guidelines for registration...
 - (5) US-EPA: Pesticide assessment guidelines, Subdivision N. Chemistry: Environmental fate (1982)....
 - (6) SETAC-Europe publication. (1995). Procedures for assessing the environmental fate and...
 - (7) OECD Test Guidelines Programme. (1995). Final Report of the OECD...
 - (8) ISO/DIS 5667-12. (1994). Water quality - Sampling - Part 12:...
 - (9) US-EPA (1998a). Sediment/water microcosm biodegradation test. Harmonised Test Guidelines (OPPTS...
 - (10) DFG: Pesticide Bound Residues in Soil. Wiley-VCH (1998).
 - (11) T.R. Roberts: Non-extractable pesticide residues in soils and plants. Pure...
 - (12) OECD Test Guideline 304A: Inherent Biodegradability in Soil (adopted 12...
 - (13) OECD (1993): Guidelines for Testing of Chemicals. Paris. OECD (1994-2000):...
 - (14) Scholz, K., Fritz R., Anderson C. and Spiteller M. (1988)...
 - (15) Guth, J.A. (1981). Experimental approaches to studying the fate of...
 - (16) Madsen, T., Kristensen, P. (1997). Effects of bacterial inoculation and...
 - (17) Steber, J., Wierich, P. (1987). The anaerobic degradation of detergent...
 - (18) Black, C.A. (1965). Methods of Soil Analysis. Agronomy Monograph No....
 - (19) APHA (1989). Standard Methods for Examination of Water and Wastewater...

- (20) Rowell, D.L. (1994). Soil Science Methods and Applications. Longman.
- (21) Light, T.S. (1972). Standard solution for redox potential measurements. Anal....
- (22) SETAC-Europe publication (1991). Guidance document on testing procedures for pesticides...
- (23) SETAC-Europe publication. (1993). Guidance document on sediment toxicity tests and...
- (24) Vink, J.P.M., van der Zee, S.E.A.T.M. (1997). Pesticide biotransformation in...
- (25) Vink, J.P.M., Schraa, G., van der Zee, S.E.A.T.M. (1999). Nutrient...
- (26) Anderson, T.H., Domsch, K.H. (1985). Maintenance carbon requirements of actively-metabolising...
- (27) ISO-14240-2. (1997). Soil quality - Determination of soil microbial biomass...
- (28) Beelen, P. Van and F. Van Keulen. (1990), The Kinetics...
- (29) Shelton, D.R. and Tiedje, J.M. (1984). General method for determining...
- (30) Birch, R.R., Biver, C, Campagna, R., Gledhill, W.E., Pagga, U.,...
- (31) Pagga, U. and Beimborn, D.B. (1993). Anaerobic biodegradation tests for...
- (32) Nuck, B.A. and Federle, T.W. (1986). A batch test for...
- (33) US-EPA (1998b). Anaerobic biodegradability of organic chemicals. Harmonised Test Guidelines...
- (34) Sijm, Haller and Schrap (1997). Influence of storage on sediment...
- (35) Timme, G., Frehse H. and Laska V. (1986) Statistical interpretation...
- (36) Timme, G., Frehse, H. (1980) Statistical interpretation and graphic representation...
- (37) Carlton, R.R. and Allen, R. (1994). The use of a...

ANNEX 1 GUIDANCE ON THE AEROBIC AND THE ANAEROBIC TEST SYSTEMS

- Aerobic test system
- Anaerobic test system

ANNEX 2 EXAMPLE OF A GAS FLOW-THROUGH APPARATUS

ANNEX 3 EXAMPLE OF A BIOMETER APPARATUS

ANNEX 4 EXAMPLE CALCULATION FOR APPLICATION DOSE TO TEST VESSELS

Status: EU Directives are being published on this site to aid cross referencing from UK legislation. After IP completion day (31 December 2020 11pm) no further amendments will be applied to this version.

- (1) [OJ 196,16.8.1967, p. 1](#). Directive as last amended by Commission Directive 2001/59/EC ([OJ L 225, 6.8.2001, p. 1](#))
- (2) [OJ L 358, 18.12.1986, p. 1](#). Directive as last amended by Directive 2003/65/EC of the European Parliament and of the Council ([OJ L 230,16.9.2003, p. 32](#)).