[Form 6] Report on the Results of the Chromosome Aberration Test using Cultured Mammalian Cells

1. General information

Name of new chemical substance (based on the IUPAC nomenclature system)			
Other name			
CAS no.			
Structural or rational formula (if neither is available, summarize its formulation method)			
Molecular weight			
Purity of the new chemical substance used for the test (%)			
Lot number of the new chemical substance used for the test			
Names and contents of impurities			
Vapor pressure			
Solubility in water			
1-Octanol/water partition coefficient			
Melting point			
Boiling point			
Properties at room temperature			
Stability			
Solubility in solvents, etc.	Solvent	Solubility	Safety in solvent

[Notes] Provide the physicochemical properties wherever possible.

1. Fill in the “Vapor pressure” column with the vapor pressure of the test substance.
2. Fill in the “Stability” column with the stability of the test substance against temperature, light, etc.
3. Fill in the “Solubility in solvents, etc.” column with the solubility and stability of the test substance in a solvent.

2. Type of cells – culture conditions

Cell name		Place it was obtained from		
Type		Date it was obtained		
Culture solution		Manufacturer		
Type of blood serum and amount added	%	Manufacturer (Lot No.)		
Cell cycle	h	Freezing conditions		
Passage number		Culture conditions	Container	
Number of chromosomes (Mode)	chromosomes		Temperature	
			CO_2 concentration	
Remarks				

3. S9 mix

(1) Method, etc. of obtaining S9 (Circle the appropriate number and record the necessary information.)

Self-made or purchased	1. Self-made 2. Purchased (manufacturer)
Date of manufacture	Manufactured on (day) (month) (year)
If purchased, the Lot No.	
Storage temperature	℃

(2) S9 preparation method

Animals used		Inducing substances	
Type and species		Name	
Sex		Method of administration	
Age in weeks	Weeks	Administration period and administered dose (g/kg weight)	
Body weight	g		

(3) Composition of S9 mix

Ingredient	Amount in 1ml of S9 mix	Ingredient	Amount in 1ml of S9 mix
S9	ml	NADP	μmol
$MgCl_2$	μmol	Na-Phosphoric acid buffering solution	μmol
KCl	μmol	Other ( )	μmol
Glucose-6-phosphoric acid	μmol		

4. Preparation of the test substance solution (Circle the appropriate numbers concerning the properties of the test substance solution and whether or not there is purity conversion.)

Solvent used	Name	Manufacturer	Lot No.	Grade	Purity (%)
Reason for selection of solvent					
Properties of the test substance solution	Dissolution Suspension Other ( )				
Methods for suspension, etc. when the test substance is very insoluble					
Solution storage time and temperature from the time of preparation to the time of use	hours minutes ℃				
Is there purity conversion?	Yes No				

5. Test using short-term treatment process

(1) Cell growth inhibition test conditions

		When not using the metabolic activation method	When using the metabolic activation method
Test implementation period		From (day) (month) (year) to (day) (month) (year)	From (day) (month) (year) to (day) (month) (year)
Incubator	Shape		
	Size		
	Amount of culture solution	ml/incubator	ml/incubator
	No. of incubators per dosage		
Cell	No. of disseminated cells	cells/ml	cells/ml
	No. of days of advance cultivation	days	days
Treatment conditions	Amount of test substance solution added	ml/incubator	ml/incubator
	Amount of S9 mix added		ml/incubator
	Final concentration of S9		
	Final concentration of S9 protein		
	Treatment period	h	h
	Recovery period	h	h
Cell growth inhibition measurement method			
Remarks			

(2) Cell growth inhibition test results

When not using the metabolic activation method (- h)		When using the metabolic activation method (- h)	
Dosage (mg/ml)	Cell growth index (%)	Dosage (mg/ml)	Cell growth index (%)

[Note]

Record the treatment period and recovery period in parentheses.

Make the cell growth index of the group treated with a solvent 100% and record in order of ascending concentration.

(3) Chromosome aberration test conditions

		When not using the metabolic activation method	When using the metabolic activation method
Test implementation period		From (day) (month) (year) to (day) (month) (year)	From (day) (month) (year) to (day) (month) (year)
Incubator	Shape		
	Size		
	Amount of culture solution	ml/incubator	ml/incubator
	No. of incubators per dosage		
Cell	No. of disseminated cells	cells/ml	cells/ml
	No. of days of advance cultivation	days	days
Treatment conditions	Amount of test substance solution added	ml/incubator	ml/incubator
	Amount of S9 mix added		ml/incubator
	Final concentration of S9		
	Final concentration of S9 protein		
	Treatment period	h	h
	Recovery period	h	h
Remarks			

(4) Chromosome aberration test results (in Appendix 1)

6. Test using continuous treatment process (implement this test if the test using short-term treatment process is judged to have given a negative result)

(1) Cell growth inhibition test conditions

Test implementation period		From (day) (month) (year) to (day) (month) (year)	From (day) (month) (year) to (day) (month) (year)
Incubator	Shape		
	Size		
	Amount of culture solution	ml/incubator	ml/incubator
	No. of incubators per dosage		
Cell	No. of disseminated cells	cells/ml	cells/ml
	No. of days of advance cultivation	days	days
Treatment conditions	Amount of test substance solution added	ml/incubator	ml/incubator
	Treatment period	h	h
	Recovery period	h	h
Cell growth inhibition measurement method			
Remarks			

(2) Cell growth inhibition test results

When using (- h) treatment		When using (- h) treatment	
Dosage (mg/ml)	Cell growth index (%)	Dosage (mg/ml)	Cell growth index (%)

[Note]

Record the treatment period and recovery period in parentheses.

The continuous treatment process uses a method that does not depend on metabolic activation.

Make the cell growth index of the group treated with a solvent 100% and record in order of ascending concentration.

(3) Chromosome aberration test conditions

Test implementation period		From (day) (month) (year) to (day) (month) (year)	From (day) (month) (year) to (day) (month) (year)
Incubator	Shape		
	Size		
	Amount of culture solution	ml/incubator	ml/incubator
	No. of incubators per dosage		
Cell	No. of disseminated cells	cells/ml	cells/ml
	No. of days of advance cultivation	days	days
Treatment conditions	Amount of test substance solution added	ml/incubator	ml/incubator
	Treatment period	h	h
	Recovery period	h	h
Remarks			

(4) Chromosome aberration test results (in Appendix 2)

7. Judgment of the results and reference items

(1) Judgment of the results

Judgment (Circle one.)		Positive		Negative	
Reason for judgment					
D_{20} value	Structural aberration	Short-term treatment process	-S9 mix	- h treatment	mg/ml
			+S9 mix	- h treatment	mg/ml
		Continuous treatment process		- h treatment	mg/ml
				- h treatment	mg/ml
	Numerical aberration	Short-term treatment process	-S9 mix	- h treatment	mg/ml
			+S9 mix	- h treatment	mg/ml
		Continuous treatment process		- h treatment	mg/ml
				- h treatment	mg/ml

[Note] The D_{20} value is the estimated dosage of the test substance that is necessary to induce an aberration in 20% of metaphase cells. In series of tests judged positive, record it for each type of aberration.

(2) Reference items

[Note] Record the views, etc. concerning the test results of the person responsible for the test in the “Reference items” field.

8. Other

Testing agency	Name	
	Address	Tel: Fax:
Test director	Name and status	
	Years of experience	
Test number		
Test period	From (month) (day) (year) to (month) (day) (year)	

[Notes]

1. Fill in the present form by transcribing from the final report.
2. Fill in the test number reported in the final report.
3. In the margin of this form, provide the name and affiliation of the person in charge of filling in this form.

Appendix 1 Chromosome Aberration Test Results (short-term treatment process)

Name of test substance

Treatment period (h)	S9 mix	Dosage of test substance (mg/ml)	Number of cells showing structural chromosome aberration (incidence, %)							Number of gap appearances	Cell growth index (%)	Number of cells showing numerical chromosome aberration (incidence, %)			
			Number of cells observed	Chromatid break	Chromatid exchange	Chromosome break	Chromosome exchange	Other	Total number of aberrations (%)			Number of cells observed	Polyploid	Others	Total number of aberrant cells (%)
-	-	Negative control ( )													
									( )						( )
-	-														
									( )						( )
-	-														
									( )						( )
-	-	Positive control ( )													
									( )						( )
-	+	Negative control ( )													
									( )						( )
-	+														
									( )						( )
-	+														
									( )						( )
-	+	Positive control ( )													
									( )						( )

[Remarks]

1. Record the treatment period and the recovery period in that order in the “Treatment period” column.
2. Record the dosages of the test substance in ascending order.
3. Record the solvent and the negative control substance in the parentheses. Record the names of substances shown in abbreviated form in the margin.
4. Record the data for each plate of each group in the first and second lines and then record the total in the third line.
5. If precipitation of the test substance is confirmed, mark that dosage with a †.
6. When noting the dosage for which observation of the chromosomes was disabled due to cell toxicity, record TOX in the “Number of cells observed” column.
7. When using the “Others” column, record the details in the margin.

Appendix 2 Chromosome aberration test results (continuous treatment process)

Name of test substance

Treatment period (h)	Dosage of test substance (mg/ml)	Number of cells showing structural chromosome aberration (incidence, %)							Number of gap appearances	Cell growth index (%)	Number of cells showing numerical chromosome aberration (incidence, %)			
		Number of cells observed	Chromatid break	Chromatid exchange	Chromosome break	Chromosome exchange	Other	Total number of aberrations (%)			Number of cells observed	Polyploid	Others	Total number of aberrant cells (%)
-	Negative control ( )													
								( )						( )
-														
								( )						( )
-														
								( )						( )
-	Positive control ( )													
								( )						( )
-	Negative control ( )													
								( )						( )
-														
								( )						( )
-														
								( )						( )
-	Positive control ( )													
								( )						( )

[Remarks]

1. Record the treatment period and the recovery period in that order in the “Treatment period” column.
2. Record the dosages of the test substance in ascending order.
3. Record the solvent and the negative control substance in the parentheses. Record the names of substances shown in abbreviated form in the margin.
4. Record the data for each plate of each group in the first and second lines and then record the total in the third line.
5. If precipitation of the test substance is confirmed, mark that dosage with a †.
6. When noting the dosage for which observation of the chromosomes was disabled due to cell toxicity, record TOX in the “Number of cells observed” column.
7. When using the “Others” column, record the details in the margin.