MR 300457

HUNTSMAN

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Nov 22, 2006

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Office of Pollution Prevention and Toxics
Environmental Protection Agency
1200 Pennsylvania Ave, NW
Washington, DC. 20460-0001

Contains No CBI

Attn: 8(d) Health and Safety Reporting Rule (Notification/Reporting)

Re: Submittal of PAIR Health & Safety Studies Pursuant to 40 CFR 716.21 (FR Aug 16, 2006)

Dear Sir,

Please find enclosed the requested health and safety studies relative to the PAIR request as notified in the Federal Register on August 16, 2006.

Enclosed are studies concerning:

CASNr	Description
2210-79-9	Oxirane, [(2-methylphenoxy)methyl-
5026-74-4	Oxiranemethanamine,(oxiranylmethoxy)phenyl]-N-(oxiranylmethyl)-

A IUCLID robust summary and copies of the health & safety studies are submitted herein.

Huntsman appreciates EPA's attention to this matter. Please contact the undersigned if you have any questions or require further information regarding this submission.

Sincerely,

Philip Anderson, Ph.D.
Regulatory Compliance Specialist
Advanced Materials
Huntsman LLC
Phone: 281-719-4599
Fax: 281-719-4011

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IUCLID

Data Set

Existing Chemical	:	ID: 5026-74-4
CAS No.	:	5026-74-4
EINECS Name	:	p-(2,3-epoxypropoxy)-N,N-bis(2,3-epoxypropyl)aniline
EC No.	:	225-716-2
Molecular Formula	:	C15H19NO4

Producer related part

Company	:	Huntsman (Europe) BVBA (ehemals ICI Polyurethanes)
Creation date	:	16.11.2006

Substance related part

Company	:	Huntsman (Europe) BVBA (ehemals ICI Polyurethanes)
Creation date	:	16.11.2006
Status	:	
Memo	:	
Printing date	:	23.11.2006
Revision date	:	
Date of last update	:	23.11.2006
Number of pages	:	27
Chapter (profile)	:	Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile)	:	Reliability: without reliability, 1, 2, 3, 4
Flags (profile)	:	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 APPLICANT AND COMPANY INFORMATION

Type	:	manufacturer
Name	:	Huntsman Corporation (formerly Vantico)
Contact person	:	Dr. Philip Anderson
Date	:	
Street	:	10003 Woodloch Forest Drive
Town	:	77381 The Woodlands, TX
Country	:	United States
Phone	:	
Telefax	:	
Telex	:	
Cedex	:	
Email	:	philip_anderson@huntsman.com
Homepage	:	

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1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR

1.0.3 IDENTITY OF RECIPIENTS

1.0.4 DETAILS ON CATEGORY/TEMPLATE

1.1.0 SUBSTANCE IDENTIFICATION

1.1.1 GENERAL SUBSTANCE INFORMATION

Purity type	:	typical for marketed substance
Substance type	:	organic
Physical status	:	liquid
Purity	:	> 99 % v/v
Colour	:	clear to slightly amber
Odour	:	
Remark	:	monomer content > 95%

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1.1.2 SPECTRA

1.2 SYNONYMS AND TRADENAMES

Araldite MY 0510

15.11.2006

oxiranemethanamine, N-[4-(oxiranylmethoxy)phenyl]-N-(oxiranylmethyl)-

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1.3 IMPURITIES

Purity : typical for marketed substance
CAS-No :
EC-No :
EINECS-Name :
Molecular formula :
Value :

Remark : Specifications: hydrolyzable chloride = 0-2000 ppm
20.11.2006

1.4 ADDITIVES

1.5 TOTAL QUANTITY

1.6.1 LABELLING

1.6.2 CLASSIFICATION

1.6.3 PACKAGING

1.7 USE PATTERN

Type of use : type
Category : Non dispersive use

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Type of use : use
Category : Adhesive, binding agents

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Type of use : use
Category : other: almost exclusively used by Aerospace engineering and construction

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1.7.1 DETAILED USE PATTERN

1.7.2 METHODS OF MANUFACTURE

1.8 REGULATORY MEASURES

1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES

1.8.2 ACCEPTABLE RESIDUES LEVELS

1.8.3 WATER POLLUTION

1.8.4 MAJOR ACCIDENT HAZARDS

1.8.5 AIR POLLUTION

1.8.6 LISTINGS E.G. CHEMICAL INVENTORIES

1.9.1 DEGRADATION/TRANSFORMATION PRODUCTS

1.9.2 COMPONENTS

1.10 SOURCE OF EXPOSURE

1.11 ADDITIONAL REMARKS

1.12 LAST LITERATURE SEARCH

1.13 REVIEWS

2.1 MELTING POINT

2.2 BOILING POINT

2.3 DENSITY

Type	:	density
Value	:	1.21 - 1.22 g/cm³ at 25 °C
Method	:	
Year	:	
GLP	:	
Test substance	:	as prescribed by 1.1 - 1.4

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2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

2.5 PARTITION COEFFICIENT

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

2.6.2 SURFACE TENSION

2.7 FLASH POINT

2.8 AUTO FLAMMABILITY

2.9 FLAMMABILITY

2.10 EXPLOSIVE PROPERTIES

2.11 OXIDIZING PROPERTIES

2.12 DISSOCIATION CONSTANT

2.13 VISCOSITY

2.14 ADDITIONAL REMARKS

3.1.1 PHOTODEGRADATION

3.1.2 STABILITY IN WATER

3.1.3 STABILITY IN SOIL

3.2.1 MONITORING DATA

3.2.2 FIELD STUDIES

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

3.3.2 DISTRIBUTION

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Type	:	aerobic
Inoculum	:	activated sludge, domestic
Concentration	:	19 mg/l related to Test substance 12 mg/l related to DOC (Dissolved Organic Carbon)
Contact time	:	28 day(s)
Degradation	:	3.4 (±) % after 28 day(s)
Result	:	under test conditions no biodegradation observed
Kinetic of testsubst.	:	9 day(s) 1 % 14 day(s) 1 % 19 day(s) 1 % 27 day(s) 2.1 % 29 day(s) 3.4 %
Control substance	:	Acetic acid, sodium salt
Kinetic	:	9 day(s) 70.5 % 29 day(s) > 100 %
Deg. product	:	
Method	:	OECD Guide-line 301 B "Ready Biodegradability: Modified Sturm Test (CO_2 evolution)"
Year	:	1992
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	Milli-Q water was added to the weighing bottle containing the test substance and mixed by by ultra sonification; the resulting mixture was added to the test media. The test solutions were stirred continuously during the test.
Reliability	:	(1) valid without restriction
Flag	:	confidential, Critical study for SIDS endpoint

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3.6 BOD5, COD OR BOD5/COD RATIO

3.7 BIOACCUMULATION

3.8 ADDITIONAL REMARKS

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	static
Species	:	Cyprinus carpio (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC0	:	3.2
LC50	:	4.2
LC100	:	5.6
Limit test	:	
Analytical monitoring	:	no
Method	:	OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year	:	1992
GLP	:	no
Test substance	:	
Test condition	:	Hardness: 250 mg/l CaCO3 pH: 8.0 +/-0.2 Dissolved O2: 6.7 - 9.2 mg/l Temperature: 20.4 - 20.5 degree C Illumination: 16 hours photoperiod/day 7 fish per concentration and controls Reference substance: pentachlorophenol Test substance concentrations: 1.0, 1.8, 3.2, 5.6, 10.0 mg/l Acetone was used as a vehicle.
Reliability	:	(1) valid without restriction
Flag	:	confidential, Critical study for SIDS endpoint

23.11.2006 (3)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

Type	:	aquatic
Species	:	Pseudomonas putida (Bacteria)
Exposure period	:	16 hour(s)
Unit	:	mg/l
EC10	:	> 10
Analytical monitoring	:	no
Method	:	DIN 38412, part8
Year	:	1991
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	Suspensions of Pseudomonas were exposed to concentrations of test substance ranging from 312.5 - 10,000 ug/l. Acetone was used as a vehicle. Reference substance was 3,5-dichlorophenol. Temperature: 23 +/- 1 degree C.
Result	:	No toxicity was observed at any concentration of test substance. EC50 of reference substance = 20 mg/l.
Reliability	:	(1) valid without restriction

Flag : confidential, Critical study for SIDS endpoint
23.11.2006 (4)

4.5.1 CHRONIC TOXICITY TO FISH

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS

4.6.4 TOX. TO OTHER NON MAMM. TERR. SPECIES

4.7 BIOLOGICAL EFFECTS MONITORING

4.8 BIOTRANSFORMATION AND KINETICS

4.9 ADDITIONAL REMARKS

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD50
Value	:	1037 mg/kg bw
Species	:	rat
Strain	:	other: Tif- RAIf (SPF)
Sex	:	male/female
Number of animals	:	10
Vehicle	:	other: polyethylene glycol
Doses	:	400, 1000, 2000, 3000 mg/kg bw
Method	:	other: similar to Directive 84/449/EEC, B.1
Year	:	
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	5 males and 5 females per dose group were fasted overnight and treated by a single oral intubation of the test substance. Physical condition and rate of deaths were monitored throughout the 14 day observation period. All animals were necropsied at death or at the end of the study. LD50 and 95% confidence limits were calculated by the logit model.
Result	:	The LD50 95% confidence limits are 648-1403 mg/kg bw. Clinical changes observed were sedation, dyspnea, ruffled fur, exophthalmos, and curved body position. The surviving animals recovered within 9-12 days. Necropsy revealed no substance-related gross organ changes. Deaths recorded were

Dose:	males:	females:
400	0 /5	0 /5
1000	2 /5	4 /5
2000	4 /5	4 /5
3000	5 /5	5 /5

Reliability	:	(2) valid with restrictions
Flag	:	confidential, Critical study for SIDS endpoint

20.11.2006 (5)

Type	:	LD50
Value	:	1413 mg/kg bw
Species	:	mouse
Strain	:	other: Tif- MAG (SPF)
Sex	:	male/female
Number of animals	:	10
Vehicle	:	other: polyethylene glycol
Doses	:	600, 1000, 1500, 2500, 3000 mg/kg bw
Method	:	other: similar to Directive 84/449/EEC, B.1
Year	:	
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	5 males and 5 females per dose group were fasted overnight and treated by a single oral intubation of the test substance. Physical condition and rate of deaths were monitored throughout the 14 day observation period. All animals were necropsied at death or at the end of the study. LD50 and 95% confidence limits were calculated by the logit model.
Result	:	The LD50 95% confidence limits are 1024-1873 mg/kg bw.

Clinical changes observed were sedation, dyspnea, ruffled fur, exophthalmos, and curved body position. The surviving animals recovered within 9 days.
Deaths recorded were:

Dose:	males:	females:
600	0 /5	1 /5
1000	1 /5	0 /5
1500	4 /5	3 /5
2500	4 /5	4 /5
3000	4 /5	5 /5

Reliability : (2) valid with restrictions
Flag : confidential, Critical study for SIDS endpoint
20.11.2006 (6)

Type : LD50
Value : 2739 mg/kg bw
Species : Chinese hamster
Strain :
Sex : male/female
Number of animals : 10
Vehicle : other: polyethylene glycol
Doses : 1000, 2000, 2500, 3000, 5000 mg/kg bw
Method : other: similar to Directive 84/449/EEC, B.1
Year :
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Method : 5 males and 5 females per dose group were fasted overnight and treated by a single oral intubation of the test substance. Physical condition and rate of deaths were monitored throughout the 14-18 day observation period. All animals were necropsied at death or at the end of the study.
LD50 and 95% confidence limits were calculated by the logit model.
Result : The LD50 95% confidence limits are 2276-3429 mg/kg bw.
Clinical changes observed were sedation, dyspnea, ruffled fur, exophthalmos, and curved body position. The surviving animals recovered within 7-18 days. Necropsy revealed no substance-related gross organ changes.
Deaths recorded were

Dose:	males:	females:
1000	0 /5	0 /5
2000	0 /5	0 /5
2500	2 /5	0 /5
3000	3 /5	5 /5
5000	5 /5	5 /5

Reliability : (2) valid with restrictions
Flag : confidential, Critical study for SIDS endpoint
20.11.2006 (7)

Type : LD50
Value : 1000 - 3000 mg/kg bw
Species : rat
Strain :
Sex : male
Number of animals : 1
Vehicle : other: propylene glygol
Doses : 30, 100, 300, 1000, 3000, 10000 mg/kg bw
Method : other: similar to EPA OTS 798.1175
Year :
GLP : no
Test substance : other TS

Method : One male rat per dose level was administered the test substance as a

suspension in PEG or undiluted at 3000 and 10000 mg./kg bw.

Result : Hypoactivity and ruffed fur were noted in all animals within 15 minutes post-treatment. Diarrhea was noted in animals at 3000 and 10000 mg/kg dose levels within 2 hours. All surviving animals recovered within 6-22 hours. Necropsy of the animals that died revealed severe gastroenteritis. Necropsy of the survivors at the end of the 14 day observation period revealed red, mottled lungs in one animal and no other gross pathologic alterations.
Deaths recorded:

Dose:	males:
30	0/1
100	0/1
300	0/1
1000	0/1
3000	1/1
10000	1/1

Test substance : Epoxy 0500:
purity = > 95%
monomer content: 62-75%
dimer content: 9-19%
Impurities : epichlorhydrin = 0 to150 ppm; hydrolyzable chloride = 300 to 3000 ppm

Reliability : (2) valid with restrictions
Flag : confidential, Critical study for SIDS endpoint
20.11.2006 (8)

5.1.2 ACUTE INHALATION TOXICITY

Type : LC50
Value : > 46.2 mg/m³
Species : rat
Strain : other: Charles River
Sex : male/female
Number of animals : 10
Vehicle : other: undilted in air
Doses : 46.2 mg/m3
Exposure time : 4 hour(s)
Method : other: similar to EPA OTS 798.1150
Year :
GLP : no
Test substance : other TS

Result : There was no mortality of test animals.
Animals were hypoactive during the exposure period. Average body weight gains during the 14 day observation period were within normal limits. Necropsy, performed on all animals at the end of the observation period, revealed no gross pathologic conditions.
Test condition : the concentrtationtested was the maximum attainable at ambient conditions.
Test substance : Epoxy 0500:
purity = > 95%
monomer content: 62-75%
dimer content: 9-19%
Impurities : epichlorhydrin = 0 to150 ppm; hydrolyzable chloride = 300 to 3000 ppm
Reliability : (2) valid with restrictions
Flag : confidential, Critical study for SIDS endpoint
20.11.2006 (9)

5.1.3 ACUTE DERMAL TOXICITY

Type	:	LD50
Value	:	> 4000 mg/kg bw
Species	:	rat
Strain	:	other: Tif- RAIf (SPF)
Sex	:	male/female
Number of animals	:	10
Vehicle	:	other: neat
Doses	:	3000, 3500, 4000 mg/kg bw
Method	:	other: similar to Directive 92/69/EEC, B.3
Year	:	
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	The backs of 5 males and 5 females rats per dose group were shaved; 24 hours later the test material was dispersed with a syringe and covered with an occlusive dressing fastened with an adhesive elastic bandage. After 24 hours, the dressing was removed and the skin cleaned with warm water. The reaction of the skin was noted. Physical condition and rate of deaths were monitored throughout the 14 day observation period. All animals were necropsied at death or at the end of the study. LD50 and 95% confidence limits were calculated by the logit model.
Result	:	Clinical changes observed were sedation, dyspnea, ruffled fur, exophthalmos, curved body position, and erthema at treatment site. All animals recovered within 9 days. No deaths were observed at any dose level.
Reliability	:	(2) valid with restrictions
Flag	:	confidential, Critical study for SIDS endpoint

20.11.2006 (10)

Type	:	LD50
Value	:	> 3000 - mg/kg bw
Species	:	rabbit
Strain	:	New Zealand white
Sex	:	male
Number of animals	:	3
Vehicle	:	other: neat
Doses	:	300, 1000, 3000 mg/kg bw
Method	:	other: similar to EPA OTS 798.1100
Year	:	
GLP	:	no
Test substance	:	other TS
Result	:	No pharmacotoxic symptoms were noted in any test animals. The test substance was moderately-severely irritating to the skin of albino rabbits. Necropsy revealed red lungs in all rabbits. No other gross pathology was noted except for local skin reactions.
Test condition	:	The skin at the application site was abraded.
Test substance	:	Epoxy 0500: purity = > 95% monomer content: 62-75% dimer content: 9-19% Impurities : epichlorhydrin = 0 to150 ppm; hydrolyzable chloride = 300 to 3000 ppm
Reliability	:	(2) valid with restrictions
Flag	:	confidential, Critical study for SIDS endpoint

20.11.2006 (11)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

5.2.1 SKIN IRRITATION

Species	:	rabbit
Concentration	:	undiluted
Exposure	:	Occlusive
Exposure time	:	24 hour(s)
Number of animals	:	6
Vehicle	:	other: neat
PDII	:	4.1
Result	:	moderately irritating
Classification	:	irritating
Method	:	other: US EPA Sec. 163.81-5: Primary dermal irritation study
Year	:	1978
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	US EPA Sec. 163.81-5: Primary dermal irritation study. August 22, 1978. Federal Register. Vol. 43 No. 163. Before treatment he backs of 6 males were shaved and immediately before application one side of the back was scarified. The test material (0.5ml) was added to a gauze patch and applied to both intact and abraded skin. The patches were covered with an impermeable material and fastened with adhesive tape. After 24 hours, the dressing was removed and the reaction of the skin was noted. The skin reaction was noted throughout the 7 day observation period.
Result	:	The test substance produced a marked irritation when applied to intact and abraded rabbit skin. The primary irritation index was 4.7/8.0
Reliability	:	(2) valid with restrictions
Flag	:	confidential, Critical study for SIDS endpoint

20.11.2006 (12)

5.2.2 EYE IRRITATION

Species	:	rabbit
Concentration	:	undiluted
Dose	:	.1 ml
Exposure time	:	30 seconds
Comment	:	Rinsed and not rinsed
Number of animals	:	6
Vehicle	:	none
Result	:	slightly irritating
Classification	:	irritating
Method	:	US EPA Sec. 163.81-5: Primary eye irritation study. August 22, 1978. Federal Register. Vol. 43 No. 163.
Year	:	1978
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	Mean primary irritation index: 3.3/110. The primary irritation index: for unrinsed eyes = 5.5; for rinsed eyes = 1.2
Reliability	:	(2) valid with restrictions
Flag	:	confidential, Critical study for SIDS endpoint

16.11.2006 (13)

Species	:	rabbit
Concentration	:	undiluted
Dose	:	.1 ml
Exposure time	:	
Comment	:	not rinsed
Number of animals	:	3
Vehicle	:	none
Result	:	moderately irritating
Classification	:	irritating
Method	:	Draize Test
Year	:	
GLP	:	no
Test substance	:	other TS: Epoxy 0500
Result	:	Irritation score: 32.7/110
Test substance	:	Epoxy 0500: monomer content: 62-75% dimer content: 9-19%
Reliability	:	(2) valid with restrictions
Flag	:	confidential, Critical study for SIDS endpoint

16.11.2006 (23)

5.3 SENSITIZATION

Type	:	Maurer optimisation test
Species	:	guinea pig
Concentration	:	1st: Induction .1 % intracutaneous 2nd: Challenge .1 % intracutaneous 3rd: Challenge 5 % occlusive epicutaneous
Number of animals	:	20
Vehicle	:	other: propylene glycol
Result	:	sensitizing
Classification	:	sensitizing
Method	:	other: similar to the "appraisal of Safety of Chemicals in Foods, Drugs, Cosmetics. US Food and Drug Officials
Year	:	1959
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4

Method :

During the induction period, the animals received one injection every second day (except weekends) to a total of 10 injections of freshly prepared 0.1% dilution of test substance in propylene glycol. One control group was treated with vehicle only. 24 hours later the skin reaction was assessed by measuring the area of erythema and the skin-fold thickness. The 'reaction volume' was calculated from these values for each animal.

During weeks 2 and 3, six doses (0.1ml) consisting of the test substance mixed 1:1 with adjuvant (Complete Bacto Adjuvant) was injected into the skin on the neck of the animals on Monday, Wednesday and Friday.

Two weeks after the last induction dose, 0.1ml of the initial test substance formulation was injected intradermally into the untreated flank. Twenty-four hours later, the 'reaction volume' was assessed.

Ten days after the intracutaneous challenge, a subirritant dose of test compound in vaseline was applied epicutaneously under occlusion for 24 hours. The irritation was scored according to Draize assay.

A positive was defined as final 'reaction volume' exceeding the mean + 1 Std Dev of the 4 'pre-sensitizing' responses.
Statistical significance between controls and treated animals was assessed by the exact Fisher test.

Result : Incidence of positive reactions after intradermal challenge:
Vehicle: 4/20
TK12759: 8/20
Incidence of positive reactions after occlusive epicutaneous challenge:
Vehicle: 0/20
TK12759: 20/20

Reliability : (2) valid with restrictions
Flag : confidential, Critical study for SIDS endpoint
20.11.2006 (14)

5.4 REPEATED DOSE TOXICITY

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Salmonella typhimurium reverse mutation assay
System of testing : Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538; Escherichia coli WP2 uvrA
Test concentration : 5 to 5000 ug/plate
Cycotoxic concentr. :
Metabolic activation : with and without
Result : positive
Method : other: similar to Directive 84/449/EEC, B.14
Year :
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Method : The test substance was dissolved in DMSO. The activation system contained 0.1 ml of S-9 fraction of liver from rats induced with Aroclor 1264.
DMSO alone was used for the negative control.
Positive controls in the non-activated assays
for TA 98 and TA 1538: 3-nitrofluorene;
for TA 100 and TA 1535:N-ethyl-N?-nitro-N-nitrosoguanidine;
for TA 1537: 9(5) aminoacridine HCl monohydrate.
In the activated assays, the positive control was 2-aminoanthracene.
The plates, triplicates for each dose, were incubated in darkness for 48 hours at 37 degree C.
When the colonies were counted, the arithmetic mean was calculated. A positive test is defined as a doubled colony count doubled as compared to control.

Result : An increase in colony counts were seen with TA100 and TA1535 both with and without metabolic activation when treated with TK 12759. An increase in colony counts were also seen with TA98 without metabolic activation when treated with TK 12759.
Reliability : (1) valid without restriction
Flag : confidential, Critical study for SIDS endpoint
20.11.2006 (15)

Type : Mammalian cell gene mutation assay
System of testing : mouse embryo fibroblasts (Balb/3T3, clone A31-1-1)
Test concentration : 3.75, 7.5, 15, 30, 60 ug/ml
Cycotoxic concentr. : 60 ug/ml
Metabolic activation : with
Result : negative

Method	:	Directive 87/302/EEC, part B, p. 61
Year	:	
GLP	:	
Test substance	:	as prescribed by 1.1 - 1.4

Result :

concentration:	No. positive plates:	Transformation Frequency:
solvent control	3/15	1.53
untreated control	2/13	0.78
3.75 ug/ml	6/15	2.74
7.5 ug/ml	3/15	1.01
15.0 ug/ml	5/15	1.76
30.0 ug/ml	6/15	3.93
60.0 ug/ml	4/15	16.22
Positive control(50 ug/ml)	12/15	11.92
Positive control(100 ug/ml)	11/14	29.22

Conclusion	:	Statistical comparison revealed no significsnt differences between solvent control and various test concentrations.
Reliability	:	(1) valid without restriction
Flag	:	confidential, Critical study for SIDS endpoint

20.11.2006 (16)

Type	:	Mammalian cell gene mutation assay
System of testing	:	mouse embryo fibroblasts (Balb/3T3)
Test concentration	:	0.41x10e-5 to 6.5x10e-5 ul/ml
Cycotoxic concentr.	:	6.5x10e-5 ul/ml
Metabolic activation	:	without
Result	:	negative
Method	:	Directive 87/302/EEC, part B, p. 61
Year	:	
GLP	:	
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	The test substance was dissolved in DMSO. Two positive controls consisted of methylcholanthrene (1.5 and 3.0 ug/ml). Two negative controls consisted of vehicle and untreated.
Result	:	No colonies of transformed cells were detected in both negative controls and all concentrations of test substance. The transformation frequency of the positive controls (1.5 and 3.0 ug/ml) were 10.3 and 78.0, respectively.
Reliability	:	(2) valid with restrictions
Flag	:	confidential, Critical study for SIDS endpoint

20.11.2006 (17)

Type	:	Mouse lymphoma assay
System of testing	:	mouse lymphoma cells (L5178Y)
Test concentration	:	0.21 and 0.51ug/ml
Cycotoxic concentr.	:	>0.51ug/ml
Metabolic activation	:	
Result	:	positive
Method	:	Directive 87/302/EEC, part B, p. 61
Year	:	
GLP	:	
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	The cells were cultured in an agar containing anti-metabolites: methotrexate, thymidine, and cytosine arabinoside. Cell viability control contained no anti-metabolites. A 4-hour and an 18-hour incubation time was used.
Result	:	In the 4-hour assay, there was no difference in the number of forward mutant colonies between the treated and control cells after exposure to the anti-metabolites. In the 18-hour assay, the number of mutant colonies was elevated in the thymidine selection medium with a mutation factor of 2.86.
Conclusion	:	The test substance displayed a weak mutagenic activity.

Reliability	:	(1) valid without restriction
Flag	:	confidential, Critical study for SIDS endpoint
20.11.2006		(18)

Type	:	Ames test
System of testing	:	Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538 and Saccharomyces D4
Test concentration	:	0.1 to 10 ul/plate
Cycotoxic concentr.	:	10 ul/plate to TA98
Metabolic activation	:	with and without
Result	:	positive
Method	:	other: similar to EPA OTS 798.5265
Year	:	
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	Liver microsomal enzymes were prepared from Aroclor-induced rats.
Result	:	The tests were positive in TA1535, TA100 and D4 in the presence and absence of metabolic activation.
Reliability	:	(2) valid with restrictions
Flag	:	confidential, Critical study for SIDS endpoint
20.11.2006		(19)

5.6 GENETIC TOXICITY 'IN VIVO'

Type	:	Sister chromatid exchange assay
Species	:	Chinese hamster
Sex	:	male/female
Strain	:	
Route of admin.	:	gavage
Exposure period	:	single dose
Doses	:	228, 456, 912 mg/kg in 20ml/kg PEG 400
Result	:	positive
Method	:	EPA OTS 798.5385
Year	:	
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	The number of SCEs in all concentrations (6.27, 8.25, and 12.87 SCEs/cell respectively) were significantly greater than in the concurrent negative control (4.4 SCEs/cell). The positive control [dimethyl benz(a)anthracene] yielded a mean value of 9.31 SCEs/cell which was significantly greater than the negative vehicle control.
Reliability	:	(2) valid with restrictions
Flag	:	confidential, Critical study for SIDS endpoint
20.11.2006		(20)

Type	:	other: Nucleus anomaly in somatic interphase nuclei
Species	:	Chinese hamster
Sex	:	male/female
Strain	:	
Route of admin.	:	gavage
Exposure period	:	2 days
Doses	:	228, 456, 912 mg/kg bw
Result	:	positive
Method	:	other: similar to EPA OTS 798.5385
Year	:	
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4

Result	:	The percentage of cells displaying anomalies of the nucleus in all concentrations (0.9, 0.9, 1.38% respectively) were significantly greater than in the concurrent negative control (0.1%). The positive control (cyclophosphamide, 128mg/kg) yielded 13.8% cells displaying anomalies of the nucleus which was significantly greater than the negative vehicle control.
Reliability	:	(2) valid with restrictions
Flag	:	confidential, Critical study for SIDS endpoint
20.11.2006		(21)

Type	:	Mouse spot test
Species	:	mouse
Sex	:	female
Strain	:	C57BL
Route of admin.	:	i.p.
Exposure period	:	8 weeks
Doses	:	10, 60, 120 mg/kg bw
Result	:	negative
Method	:	Directive 87/302/EEC, part B, p. 82 "Mutagenicity: - Mouse spot test"
Year	:	
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4

Method	:	The test substance, in sesame oil, was administered by intraperitoneal injection to pregnant female mice (C57 BL/6J) on the 10th day after conception. There were 96 females in the treatment groups and negative and positive controls. Ethylnitroso-urea (50.0 mg/kg) was used as the positive control. The coat of the offspring was examined for recessive spots resulting from expression of recessive genes involved in coat-color determination. Statistical value was determined by Fisher's exact test.
Remark	:	Although not run under current GLP, Quality Assurance inspections were performed.
Result	:	The litter size and survival of the offspring were not affected by any dose administered.

Group	No. Offspring	% showing RS
Negative Control	226	0.89
Positive Control	324	6.17
30 mg/kg	349	0.57
60 mg/kg	290	0.69
120 mg/kg	292	0.0

Conclusion	:	Under the experimental conditions of the study, no evidence of mutagenic effects was obtained in the offspring of pregnant mice treated with Epoxy 510.
Reliability	:	(1) valid without restriction
Flag	:	confidential, Critical study for SIDS endpoint
16.11.2006		(22)

5.7 CARCINOGENICITY

5.8.1 TOXICITY TO FERTILITY

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

5.9 SPECIFIC INVESTIGATIONS

5.10 EXPOSURE EXPERIENCE

5.11 ADDITIONAL REMARKS

6.1 ANALYTICAL METHODS

6.2 DETECTION AND IDENTIFICATION

7.1 FUNCTION

7.2 EFFECTS ON ORGANISMS TO BE CONTROLLED

7.3 ORGANISMS TO BE PROTECTED

7.4 USER

7.5 RESISTANCE

8.1 METHODS HANDLING AND STORING

8.2 FIRE GUIDANCE

8.3 EMERGENCY MEASURES

8.4 POSSIB. OF RENDERING SUBST. HARMLESS

8.5 WASTE MANAGEMENT

8.6 SIDE-EFFECTS DETECTION

8.7 SUBSTANCE REGISTERED AS DANGEROUS FOR GROUND WATER

8.8 REACTIVITY TOWARDS CONTAINER MATERIAL

(1) Product data sheet - Araldite MY 0510

(2) NOTOX. Project No. 169875. 1996. Determination of 'ready' biodegradability: carbon dioxide (CO2) evolution test (Modified Sturm test) with TK 12759, Araldite MY 0510.

(3) NOTOX. Project No. 169548. 1996. 96-hour acute toxicity study in carp with TK 12759, Araldite MY 0510.

(4) NOTOX. Project No. 169706. 1996. Pseudomonas cell multiplication inhibition test with TK 12759, Araldite MY 0510.

(5) Ciba-Geigy Ltd. 1980. Project No. 800655. Report on the acute oral LD50 in the rat of TK 12759. [CD 5/103/78]

(6) Ciba-Geigy Ltd. 1980. Project No. 800654. Report on the acute oral LD50 in the mouse of TK 12759. [CD 5/103/80]

(7) Ciba-Geigy Ltd. 1980. Project No. 800656. Report on the acute oral LD50 in the Chinese hamster of TK 12759. [CD 5/103/76]

(8) Industrial Biotest Laboratories, Inc. 1975. Project No. 601-07231. Acute oral toxicity study with Epoxy 0500 in male albino rats. [CD 5/106/7]

(9) Industrial Biotest Laboratories, Inc. 1975. Project No. 663-07230. Acute vapor inhalation toxicity study with Epoxy 0500 in albino rats. [CD 5/106/11]

(10) Ciba-Geigy Ltd. 1980. Project No. 800659. Report on the acute dermal LD50 in the rat of TK 12759. [CD 5/103/82]

(11) Industrial Biotest Laboratories, Inc. 1975. Project No. 601-07231. Acute dermal toxicity study with Epoxy 0500 in male albino rabbits. [CD 5/106/8]

(12) Ciba-Geigy Ltd. 1980. Project No. 800658. Report on skin irritation in the rabbit after single application of TK 12759. [CD 5/103/75]

(13) Ciba-Geigy Ltd. 1980. Project No. 800657. Report on eye irritation in the rabbit after single application of TK 12759.

(14) Ciba-Geigy Ltd. 1980. Project No. 800660. Report on skin sensitizing (contact allergenic) effect in guinea pigs of TK 12759. [CD 5/103/93]

(15) Ciba-Geigy Ltd. 1982. Project No. 810939. Salmonella and Escherichia/liver microsome test of TK 12759. [CD 5/103/88]

(16) Ciba-Geigy Ltd. 1987. Project No. 8760921. Transformation/liver microsome test. [CD 5/107/97]

(17) Ciba-Geigy Ltd. 1982. Project No. 801549. BALB/3T3 cell transformation assay.

(18) Ciba-Geigy Ltd. 1981. Project No. 810994. Point mutation assay with mouse lymphoma cells. [CD 5/103/89]

(19) Litton Bionetics, Inc. 1978. Project No. 20838. Mutagenicity evaluation of MA-66 in the Ames Salmonella/Microsome plate test. [CD 5/103/86]

(20) Ciba-Geigy Ltd. 1981. Project No. 800653. Sister Chromatid Exchange Study with TK12759 in Chinese hamster. [CD 5/103/91]

(21) Ciba-Geigy Ltd. 1981. Project No. 800652. Nucleus anomaly in somatic interphase nuclei with TK12759 in Chinese hamster. [CD 5/103/90]

(22) Ciba-Geigy Ltd. 1987. Project No. 874067. Mammalian Spot Test, Mouse, 8 weeks. [CD 5/107/98]

(23) Industrial Biotest Laboratories, Inc. 1975. Project No. 601-07231. Eye irritation test with Epoxy 0500 in albino rabbits. [CD 5/106/9]

10.1 END POINT SUMMARY

10.2 HAZARD SUMMARY

10.3 RISK ASSESSMENT

120591

CIBA-GEIGY Limited
Basle, Switzerland

Exp. Toxicology Sisseln
GU 2.1

REPORT ON

EYE IRRITATION IN THE RABBIT AFTER SINGLE APPLICATION OF

TK 12759

Project No.:	800657
Sponsor:	Plastics and Additives Division
Test material received:	March 28, 1980
Additional identification:	Physical appearance: yellow liquid Batch No.: T60011
The experiment started:	May 19, 1980
The experiment ended:	May 26, 1980
Study director:	L. Ullmann
Approved by:	Dr. med. vet. W. Gfeller
Reported:	June 2, 1980/wu
Archives:	Sisseln WST 452

800657

1. Summary and Conclusion

Under the conditions of this experiment the test material was found to cause a minimal irritation when applied to the rabbit eye mucosa.

The primary irritation index was found to be:

unrinsed eyes	=	5.5
rinsed eyes	=	1.2
Mean irritation index for unrinsed and rinsed eyes:		3.3

2. Method

Concentration: conc.

The procedure used is described in the Proposed Guidelines of the United States Environmental Protection Agency (EPA) § 163.81-4 "Primary eye irritation study", Federal Register, Vol. 43, No. 163, August 22, 1978.

The test was performed on 6 male New Zealand White rabbits bred and raised on the premises weighing 2 to 3 kgs. The animals were housed individually in metal cages, numbered by ear tags, were kept at a constant room temperature of 22 ± 2°C, at a relative humidity of 55 ± 10 % and on a 10 hour light cycle day. The animals had ad libitum access to standard rabbit food - NAFAG, No. 814, Gossau SG - and water. Prior to treatment they were adapted to our laboratories for a minimum of 4 days. Only rabbits with normal ophthalmic findings were used for these tests.

The test material in an amount of 0.1 ml was inserted into the conjunctival sac of the left eye of the rabbits and the lids were gently closed for a few seconds. The right eye was not treated and served as an untreated control. In 3 of the 6 rabbits approximately 30 seconds after treatment the treated eye was flushed with 10 ml of physiological saline. The eye irritation was appraised with a slit-lamp on day 1, 2, 3, 4 and 7 and was scored for each individual rabbit as outlined in table 1.

800657

Score for eye irritation in rabbits (Table 1)

CORNEA

A	Opacity and degree of density (most dense area scored)	
	No opacity	0
	Scattered or diffuse area, details of iris clearly visible	1
	Easily discernible translucent areas, details of iris slightly obscured	2
	Opalescent areas, no details of iris visible, size of pupil barely discernible	3
	Opaque, iris invisible	4
B	Area of cornea involved	
	One quarter (or less) but not zero	1
	Greater than one quarter, but less than half	2
	Greater than half, but less than three quarters	3
	Greater than three quarters, up to whole area	4

A x B x 5 Maximum possible score = 80

IRIS

A	Values	
	Normal	0
	Folds above normal, congestion, swelling, circumcornea injection (any or all of these or combination of any thereof) iris still reacting to light (sluggish reaction is positive)	1
	No reaction to light, hemorrhage, gross destruction (any or all of these)	2

A x 5 Maximum possible score = 10

CONJUNCTIVAE

A	Redness (refers to palpebral and bulbar conjunctiva excluding cornea and iris)	
	Vessels normal	0
	Vessels definitely injected above normal	1
	More diffuse, deeper crimson red, individual vessels not easily discernible	2
	Diffuse beefy red	3

	Chemosis	
	No swelling	0
	Any swelling above normal (includes nictitating membrane)	1
	Obvious swelling with partial eversion of lids	2
	Swelling with lids about half closed	3
	Swelling with lids about half closed to completely closed	4
C	Discharge	
	No discharge	0
	Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
	Discharge with moistening of the lids and hairs just adjacent to lids	2
	Discharge with moistening of the lids and hairs, and considerable area around the eye	3

(A + B + C) x 2 Maximum possible score = 20

3. Calculation of the primary eye irritation index

The mean reaction scores for cornea, iris and conjunctiva observed after 1, 2, 3, 4 and 7 days were summed up and the sum divided by 5. This procedure was carried out separately for unrinsed (A) and rinsed (B) eyes respectively.

Index		
	0 - 10	= minimal
	11 - 25	= slight
	26 - 55	= moderate
	56 - 84	= marked
	above 84	= extreme

The ratio R = A : B indicates the effect of rinsing the eyes approximately 30 seconds after instillation of the compound.

<1	= rinsing increased the effect of the compound
1	= no effect
1.1 - 1.5	= practically no effect
1.6 - 2	= little but assessable effect
2.1 - 4	= good effect
4.1 - 10	= very good effect
>10	= action of the compound practically abolished

4. Results

The results of the primary eye irritation test are reported in tables 2 to 4.

TK 12759 was found to cause a minimal irritation when applied to the rabbit eye mucosa.

800657

Table 2 - Calculation of the primary eye irritation index

Time after exposure days	mean reaction score					
	unrinsed eyes (A)			rinsed eyes (B)		
	cornea	iris	conjunc-tiva	cornea	iris	conjunc-tiva
1	1.7	0	7.3	0	0	3.3
2	1.7	0	6.7	0	0	1.7
3	0	0	4	0	0	0
4	0	0	3.3	0	0	0
7	0	0	2.7	0	0	0
subtotal	3.4	0	24	0	0	6
total	27.4			6		

primary irritation index in unrinsed eyes

$$A = 27.4 : 5 = 5.5$$

primary irritation index in rinsed eyes

$$B = 6 : 5 = 1.2$$

effect of rinsing

$$R = \frac{A}{B} = \frac{5.5}{1.2} = 4.6$$

Table 3

RABBIT EYE IRRITATION SCORES - REFERENCE PROCEDURE

n = not rinsed
r = rinsed
p = purulent

	24 hours						48 hours						72 hours					
Rabbit No.:	350	351	352	353	354	355	350	351	352	353	354	355	350	351	352	353	354	355
Eye	n	n	n	r	r	r	n	n	n	r	r	r	n	n	n	r	r	r
I. Cornea																		
A Opacity	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
B Area involved	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
a = AxBx5 (Max. 80)	0	5	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0
II. Iris																		
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
b = Score x 5 (Max. 10)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
III. Conjunctiva																		
A Redness	2	2	1	2	1	0	2	2	0	2	0	0	0	2	0	0	0	0
B Chemosis	1	3	0	1	0	0	1	2	0	1	0	0	0	2	0	0	0	0
C Discharge	0	2	0	1	0	0	1	2	0	1	0	0	0	2	0	0	0	0
c= A+B+C x 2 (Max. 20)	6	14	2	8	2	0	8	12	0	8	0	0	0	12	0	0	0	0
Total = a+b+c (Max. 110)	6	19	2	8	2	0	8	17	0	8	0	0	0	12	0	0	0	0
Group Mean		9		3.3				8.3		2.7				4		0		

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Table 4

RABBIT EYE IRRITATION SCORES - REFERENCE PROCEDURE

n = not rinsed
r = rinsed
p = purulent

	4 days						7 days						days					
Rabbit No:	350	351	352	353	354	355	350	351	352	353	354	355						
Eye	n	n	n	r	r	r	n	n	n	r	r	r	n	n	n	r	r	r
I. Cornea																		
A Opacity	0	0	0	0	0	0	0	0	0	0	0	0						
B Area involved	0	0	0	0	0	0	0	0	0	0	0	0						
a = AxBx5 (Max. 80)	0	0	0	0	0	0	0	0	0	0	0	0						
II. Iris																		
	0	0	0	0	0	0	0	0	0	0	0	0						
b = Score x 5 (Max. 10)	0	0	0	0	0	0	0	0	0	0	0	0						
III. Conjunctiva																		
A Redness	0	2	0	0	0	0	0	2	0	0	0	0						
B Chemosis	0	2	0	0	0	0	0	2	0	0	0	0						
C Discharge	0	1	0	0	0	0	0	0	0	0	0	0						
c = A+B+C x 2 (Max. 20)	0	10	0	0	0	0	0	8	0	0	0	0						
Total = a+b+c (Max. 110)	0	10	0	0	0	0	0	8	0	0	0	0						
Group Mean	3.3			0			2.7			0								

800657

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REPORT

DETERMINATION OF 'READY' BIODEGRADABILITY:

CARBON DIOXIDE (CO_2) EVOLUTION TEST (MODIFIED STURM TEST)

WITH

TK 12759, ARALDITE MY 0510

NOTOX Project 169875
NOTOX Substance 59652
Ciba Project 964625

TK 12759, ARALDITE MY 0510

NOTOX Project 169875
Ciba Project 964625

REPORT APPROVAL

STUDY DIRECTOR: Ing. M.J.E. Desmares-Koopmans

.....

Date: March 27, 1996

MANAGEMENT: Ing. E.J. van de Waart
Section Head, Genetic &
Eco-Toxicology

i.a. Dr. Ilona C. Enninga
Technical Director

.....

Date: 29/03/1996

SUMMARY

TK 12759, ARALDITE MY 0510 was tested for its ready biodegradability in the Carbon Dioxide (CO_2) Evolution Test (Modified Sturm Test) at ca. 39 mg per 2 litres, corresponding to ca. 12 mg TOC/l.

The study procedure was based on EEC directive 92/69, C.4-C, December 1992, and OECD guideline No. 301 B July 17, 1992.

The Theoretical CO_2 production ($ThCO_2$) of TK 12759, ARALDITE MY 0510 (MF: $C_{15}H_{19}NO_4$, MW: 277 g) was calculated to be 2.383 mg CO_2/mg.

The relative degradation values calculated from the measurements performed during the test period revealed no significant degradation of TK 12759, ARALDITE MY 0510 in test bottle A (significant: >10%).
In test bottle B 30% degradation was measured on day 29. This result was not comparable to the results of test bottle A and the toxicity control, and it was assumed that the produced CO_2 in test bottle B was not test substance related, but the result of a technical failure. Therefore, the degradation was based on the data of day 27 and considered as not significant.

Since all further acceptability criteria were met, this study was considered to be valid.

In conclusion, TK 12759, ARALDITE MY 0510 was not readily biodegradable under the conditions of the modified Sturm test presently performed.

PREFACE

Sponsor	Ciba-Geigy Ltd. Polymers Division R-1006.520 CH-4002 BASEL Switzerland
Study Monitor	Dr. E.G. Semadeni
Testing Facility	NOTOX B.V. Hambakenwetering 3 5231 DD 's-Hertogenbosch The Netherlands
Study Director	Ing. M.J.E. Desmares-Koopmans
Technical Coordinator	Mrs. C.J. van Leeuwen-van Drunen
Study Plan	Start: February 14, 1996 Completion: March 14, 1996

TEST SUBSTANCE

Identification	TK 12759, ARALDITE MY 0510
Description	Clear yellow liquid (Determined at NOTOX)
Batch	50008M
Purity	> 95%
Test substance storage	In freezer in the dark
Stability under storage conditions	Stable
Expiry date	May 01, 1998
Stability in water	At least 96 h

The sponsor is responsible for the completeness and GLP Compliance of all test substance data.

PURPOSE

The purpose of the study is to evaluate a non-volatile test substance for its ready biodegradability in an aerobic aqueous medium with microbial activity introduced by inoculation of activated sludge.

GUIDELINES

The study procedure described in this report was based on the following guidelines:

European Economic Community (EEC), EEC directive 92/69, Part C: Methods for the determination of ecotoxicity, Publication No. L383, December 1992, C.4. "Biodegradation: determination of the 'ready' biodegradability, C.4-C: Carbon dioxide (CO_2) evolution test (Modified Sturm Test).

Organisation for Economic Co-operation and Development (OECD), OECD guidelines for Testing of Chemicals, Section 3, Degradation and Accumulation, guideline No. 301 B: "Ready Biodegradability: CO_2 Evolution Test" adopted July 17, 1992.

DEFINITIONS

Readily biodegradable: Test substances giving a result of at least 60% yield of CO_2 (within 28 days). This pass level must be reached within 10 days of biodegradation exceeding 10%.

$ThCO_2$: Theoretical carbon dioxide (mg) is the quantity of carbon dioxide calculated to be produced from the known or measured carbon content of the test substance when fully mineralized; also expressed as mg carbon dioxide evolved per mg test substance.

TOC : Total organic carbon of a sample is the sum of the organic carbon in solution and in suspension.

PREPARATION OF TEST SOLUTIONS

After addition of milli-Q water to each weighing bottle containing TK 12759, ARALDITE MY 0510 (A: 38.7 mg, B: 39.5 and toxicity control: 39.0 mg), followed by ultra sonication (15 min.), the resulting mixture was quantitatively added to the test media. The test solutions were continuously stirred during the test.

TEST SYSTEM

Source	The source of test organisms was activated sludge freshly obtained from a municipal sewage treatment plant: 'Waterschap de Maaskant', 's-Hertogenbosch, the Netherlands.
Treatment	The sludge was kept under continuous aeration until further treatment. The concentration of suspended solids was 3.2 g/l in the concentrated sludge. Before use, the sludge was allowed to settle for at least 30 minutes and the liquid decanted for use as inoculum at the amount of 10 ml/l of mineral medium.
Reason for selection	The test has been accepted internationally (EEC, OECD) for determining the 'ready' biodegradability of test substances under aerobic conditions.

TEST PROCEDURE AND CONDITIONS

Test duration	28 days (last CO_2-measurement on the 29th day).
Test vessels	2 litre all-glass brown coloured bottles.
Milli-Q water	Tap-water purified by reverse osmosis and subsequently passed over activated carbon and ion-exchange cartridges (Millipore Corp., Bedford, Mass., USA).
Stock solutions of mineral components	A) 8.50 g KH_2PO_4 21.75 g K_2HPO_4 67.20 g $Na_2HPO_4.12H_2O$ 0.50 g NH_4Cl dissolved in 1 l Milli-Q water, pH 7.4±0.2 B) 22.50 g $MgSO_4.7H_2O$ dissolved in 1 l Milli-Q water. C) 36.40 g $CaCl_2.2H_2O$ dissolved in 1 l Milli-Q water. D) 0.25 g $FeCl_3.6H_2O$ dissolved in 1 l Milli-Q water.

Mineral medium	1 l mineral medium contains: 10 ml of solution (a), 1 ml of solutions (b) to (d) and Milli-Q water.
Barium hydroxide, 0.0125 M	4 g $Ba(OH)_2.8H_2O$ per litre Milli-Q water was filtered through filter paper and stored in a sealed vessel to prevent absorption of CO_2 from the air.
CO_2-free air	A mixture of oxygen (21%) and nitrogen (79%) was led through a bottle, containing 0.5 - 1 litre 0.0125 M $Ba(OH)_2$ solution to trap CO_2 which might be present in small amounts. The CO_2-free air was sparged through the scrubbing solutions at a constant rate.
Test concentration	The test substance was tested in duplicate at ca. 39 mg per 2 litres, corresponding to ca. 12 mg TOC/l. The organic carbon content was based on the molecular formula.
Preparation of bottles:	
Pre-incubation medium	Mineral components, Milli-Q water (ca. 80% total volume) and inoculum (1% final volume) were added to each bottle. This mixture was aerated with CO_2-free air overnight to purge the system of CO_2.
Type and number of bottles	Test suspension: containing test substance and inoculum (2 bottles). Inoculum blank: containing only inoculum (2 bottles) Positive control: containing reference substance (ca. 40 mg/l sodium acetate (Merck art. 6268, batch 049 TA933768), TOC= 12 mg/l) and inoculum (1 bottle). Toxicity control: containing test substance, reference substance and inoculum (1 bottle).
Preparation	The test substance and positive control were added to the bottles. The volumes of suspensions were made up in all bottles by the addition of Milli-Q water previously aerated with CO_2-free air, resulting in the mineral medium described before. Three CO_2-absorbers (bottles filled with 100 ml 0.0125 M $Ba(OH)_2$) were connected in series to the exit air line of each test bottle.
Start of the incubation	The test was started by bubbling CO_2-free air through the solution at a rate of approximately 1-2 bubbles per second (ca. 30-100 ml/min).

DETERMINATION OF CO_2

Experimental CO_2 production	The CO_2 produced in each test bottle reacted with the barium hydroxide in the gas scrubbing bottle and precipitated out as barium carbonate. The amount of CO_2 produced was determined by titrating the remaining $Ba(OH)_2$ with 0.05 M standardized HCl.
Measurements	Titrations were made every second or third day during the first 10 days, and thereafter at least every fifth day until the 28th day. Each time the CO_2-absorber nearest to the test bottle was removed for titration; each of the remaining two absorbers was moved one position in the direction of the test bottle. A new CO_2-absorber was placed at the far end of the series. Phenolphthalein was used as pH-indicator. On the 28th day, the pH of the test suspensions was measured and 1 ml of concentrated HCl was added to each bottle. The bottles were aerated overnight to drive off CO_2 present in the test suspension. The final titration was made on day 29.
Theoretical CO_2 production	The theoretical CO_2 production was calculated from the molecular formula.

DATA EVALUATION

$ThCO_2$, expressed as mg CO_2/mg test substance, that can be generated by a test substance was calculated as follows:

$$ThCO_2 = \frac{\text{No. of carbon in test substance} \times MW\ CO_2}{MW\ \text{test substance}}$$

The first step in calculating the amount of CO_2 produced is to correct for background (endogeneous) CO_2 production. Thus the amount of CO_2 produced by a test material is determined by the difference (in ml of titrant) between the experimental and blank $Ba(OH)_2$ traps.

The amount of 0.05 N HCl titrated is converted into mg of CO_2 produced:

$$mg\ CO_2 = \frac{(0.05 \times ml\ HCl\ titrated)}{2} \times 44 = 1.1 \times ml\ of\ HCl\ titrated.$$

Calculations were based on the actual normality without rounding off. Relative degradation values was calculated from the cumulative CO_2 production relative to the total expected CO_2 production based on the total carbon content of the amount of test material present in the test bottles. They were plotted versus time together with the relative degradation of the positive control.

ACCEPTABILITY OF THE TEST

The results of the biodegradation test were considered to be valid when:

- the total CO_2 evolution in the inoculum blank at the end of the test did not normally exceed 40 mg/l. If values greater than 70 mg CO_2/l are obtained, the data and experimental technique should be examined critically.
- the difference of duplicate values for the %-degradation of the test substance at the plateau, at the end of the test or at the end of the 10-day window, as appropriate, was less than 20.
- the percentage degradation of the reference substance reached the level for ready biodegradability (60%) by 14 days.

Because of the stringency of the method, low values do not necessarily mean that the test substance is not biodegradable under environmental conditions, but indicates more work will be necessary to establish biodegradability.

Toxicity control: if less than 35% degradation (based on DOC) or less than 25% degradation (based on $ThCO_2$) occurred in 14 days, the test substance can be assumed to be inhibitory.

RESULTS

Theoretical CO_2 production

The Theoretical CO_2 production ($ThCO_2$) of TK 12759, ARALDITE MY 0510 (MF: $C_{15}H_{19}NO_4$, MW: 277 g) was calculated to be 2.383 mg CO_2/mg.

The concentration was 38.7 (A) and 39.5 mg (B) TK 12759, ARALDITE MY 0510 in 2 litres test medium. Hence, the theoretical CO_2 production following complete degradation was 92.2 mg for A and 94.1 mg per 2 litres for B.

The positive control contained 80.1 mg sodium acetate ($ThCO_2$= 1.073 mg CO_2/mg) resulting in a theoretical CO_2 production following complete degradation of 85.9 mg per 2 litres.

The toxicity control contained 80.1 mg sodium acetate and 39.0 mg TK 12759, ARALDITE MY 0510 in 2 litres of test medium. Hence, the theoretical CO_2 production following complete degradation of TK 12759, ARALDITE MY 0510 plus sodium acetate was 178.9 mg per 2 litres.

Biodegradation

The results are summarized in Tables 1-4, and Figure 1.

The relative degradation values calculated from the measurements performed during the test period revealed no significant degradation of TK 12759, ARALDITE MY 0510 in test bottle A (significant: >10%).
In test bottle B 30% degradation was measured on day 29. This result was not comparable to the results of test bottle A and the toxicity control, and it was assumed that the produced CO_2 in test bottle B was not test substance related, but the result of a technical failure. Therefore, the degradation was based on the data of day 27 and considered as not significant.

In the toxicity control more than 25% degradation occurred in 14 days (based on $ThCO_2$). Therefore, the test substance was assumed to be not inhibitory.

Monitoring of temperature and pH

The temperature recorded during the study varied between 20 and 23°C.

The pH values of the different test media were:

	Just before the start of the test:	On day 28:
Blank control (A)	7.5	7.5
Blank control (B)	7.5	7.5
Positive control	7.5	7.8
TK 12759, ARALDITE MY 0510 (A)	7.6	7.7
TK 12759, ARALDITE MY 0510 (B)	7.6	7.6
Toxicity control	7.6	7.9

Acceptability of the test

The positive control substance was degraded 79% in 14 days (see Table 1 and Figure 1).

The total CO_2 release in the blank reached a total value of 20 mg CO_2 per 2 litres of medium (see Table 4).

Except for day 29, the difference of duplicate values for %-degradation of TK 12759, ARALDITE MY 0510 was always less than 20. Since the degradation on day 29 in test bottle B was considered to be due to a technical failure, and the final judgement for this bottle was based on the data of day 27, the difference of duplicate values on day 29 had no effect on the outcome of this study.

TK 12759, ARALDITE MY 0510 was found to be not inhibitory.

CONCLUSION

TK 12759, ARALDITE MY 0510 was not readily biodegradable under the conditions of the modified Sturm test presently performed.

Table 1: CO_2 evolution and percentage of biodegradation of the positive control substance.

Day	ml titrant Blank (mean)	ml titrant CO_2 trap	Produced CO_2 in ml HCl	Normality HCl	Produced mg CO_2	Cumulative mg CO_2	Degradation %
2	49.07	40.27	8.80	0.048	9.29	9.29	10.8
5	48.90	22.88	26.02	0.048	27.48	36.77	42.8
7	47.94	33.57	14.37	0.048	15.17	51.94	60.5
9	48.88	40.73	8.15	0.048	8.61	60.55	70.5
14	47.23	40.36	6.87	0.048	7.25	67.80	78.9
19	47.90	41.48	6.42	0.049	6.92	74.72	87.0
23	48.51	41.59	6.92	0.049	7.46	82.18	95.7
27	45.60	38.15	7.45	0.049	8.03	90.21	105.0
29	43.95	42.12	1.83	0.049	1.97	92.18	107.3
29	45.20	44.80	0.40	0.049	0.43	92.61	107.8
29	48.26	48.50	0.00	0.049	0.00	92.61	107.8

Theoretical CO_2 production sodium acetate: 85.9 mg CO_2/2l

Table 2a: CO_2 evolution and percentage of biodegradation of the test substance (bottle A).

Day	ml titrant Blank (mean)	ml titrant CO_2 trap	Produced CO_2 ml HCl	Normality N	Produced mg CO_2	Cumulative mg CO_2	Degradation %
2	49.07	48.52	0.55	0.048	0.58	0.58	0.6
5	48.90	48.70	0.20	0.048	0.21	0.79	0.9
7	47.94	48.71	0.00	0.048	0.00	0.79	0.9
9	48.88	48.74	0.14	0.048	0.15	0.94	1.0
14	47.23	47.84	0.00	0.048	0.00	0.94	1.0
19	47.90	47.96	0.00	0.049	0.00	0.94	1.0
23	48.51	47.89	0.62	0.049	0.67	1.61	1.7
27	45.60	45.26	0.34	0.049	0.37	1.98	2.1
29	43.95	45.77	0.00	0.049	0.00	1.98	2.1
29	45.20	44.15	1.05	0.049	1.13	3.11	3.4
29	48.26	48.62	0.00	0.049	0.00	3.11	3.4

Theoretical CO_2 production test substance: 92.2 mg CO_2/2l

Table 2b: CO_2 evolution and percentage of biodegradation of the test substance (bottle B).

Day	ml titrant Blank (mean)	ml titrant CO_2 trap	Produced CO_2 ml HCl	Normality N	Produced mg CO_2	Cumulative mg CO_2	Degradation %
2	49.07	48.69	0.38	0.048	0.40	0.40	0.4
5	48.90	49.00	0.00	0.048	0.00	0.40	0.4
7	47.94	48.49	0.00	0.048	0.00	0.40	0.4
9	48.88	47.35	1.53	0.048	1.62	2.02	2.1
14	47.23	48.63	0.00	0.048	0.00	2.02	2.1
19	47.90	48.26	0.00	0.049	0.00	2.02	2.1
23	48.51	48.37	0.14	0.049	0.15	2.17	2.3
27	45.60	45.28	0.32	0.049	0.34	2.51	2.7
29	43.95	37.69	6.26	0.049	6.75	9.26	9.8
29	45.20	29.47	15.73	0.049	16.96	26.22	27.9
29	48.26	46.35	1.91	0.049	2.06	28.28	30.1

Theoretical CO_2 production test substance: 94.1 mg CO_2/2l

Table 3: CO_2 evolution and percentage of biodegradation of the toxicity control.

Day	ml titrant Blank (mean)	ml titrant CO_2 trap	Produced CO_2 ml HCl	Normality N	Produced mg CO_2	Cumulative mg CO_2	Degradation %
2	49.07	35.01	14.06	0.048	14.85	14.85	8.3
5	48.90	26.70	22.20	0.048	23.44	38.29	21.4
7	47.94	38.77	9.17	0.048	9.68	47.97	26.8
9	48.88	44.58	4.30	0.048	4.54	52.51	29.4
14	47.23	42.48	4.75	0.048	5.02	57.53	32.2
19	47.90	42.96	4.94	0.049	5.33	62.86	35.1
23	48.51	41.76	6.75	0.049	7.28	70.14	39.2
27	45.60	32.36	13.24	0.049	14.27	84.41	47.2
29	43.95	41.82	2.13	0.049	2.30	86.71	48.5
29	45.20	44.61	0.59	0.049	0.64	87.35	48.8
29	48.26	48.42	0.00	0.049	0.00	87.35	48.8

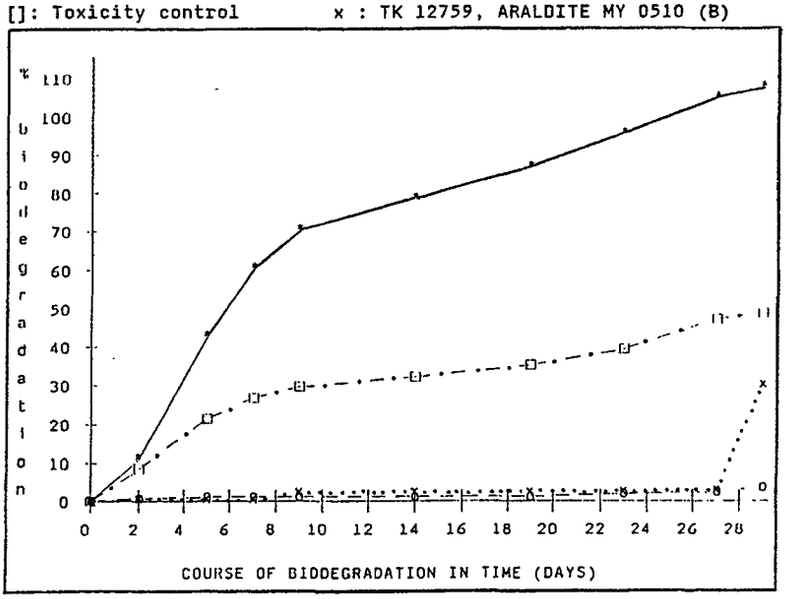
Theoretical CO_2 production test substance: 92.9 mg CO_2/2l
Theoretical CO_2 production sodium acetate: 85.9 mg CO_2/2l
Theoretical CO_2 production test substance plus sodium acetate: 178.9 mg CO_2/2l

Note: Since calculations were performed without rounding off a deviation in the last figure of the theoretical CO_2 production per 2 litres may be observed.

Table 4: CO_2 evolution in the blank.

Day	Stand. Ba(OH)2 ml HCl * 5	ml titrant Blank (mean value)	Produced CO_2 ml HCl	Normality N	Produced mg CO_2	Cumulative mg CO_2
2	9.90	49.07	0.43	0.048	0.45	0.45
5	9.81	48.90	0.15	0.048	0.16	0.61
7	9.81	47.94	1.11	0.048	1.17	1.78
9	9.83	48.88	0.27	0.048	0.29	2.07
14	9.81	47.23	1.82	0.048	1.92	3.99
19	9.84	47.90	1.30	0.049	1.40	5.39
23	9.84	48.51	0.69	0.049	0.74	6.13
27	9.83	45.60	3.55	0.049	3.83	9.96
29	9.80	43.95	5.05	0.049	5.44	15.40
29	9.80	45.20	3.80	0.049	4.10	19.50
29	9.80	48.26	0.74	0.049	0.80	20.30

Figure 1: Biodegradation of TK 12759, ARALDITE MY 0510 in the modified Sturm test:
* : Positive control
[]: Toxicity control
o : TK 12759, ARALDITE MY 0510 (A)
x : TK 12759, ARALDITE MY 0510 (B)



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J. Weiss		✓
Ablage		

REPORT

96-HOUR ACUTE TOXICITY STUDY IN CARP

WITH

TK 12759, Araldite MY 0510

NOTOX Project 169548
CIBA test number 964623

REPORT APPROVAL

STUDY DIRECTOR: Drs. M. Bogers

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Date: 24 April 1996

MANAGEMENT: Ing. E.J. van de Waart
Section Head, Genetic &
Eco-Toxicology

i.a. Dr. Ilona C. Enninga
Technical Director

.....

Date: 26/04/1996

SUMMARY

96-Hour Acute Toxicity Study in Carp with TK 12759, Araldite MY 0510.

The study procedures described in this report were based on the EEC directive 92/69, Part C.1. "Acute toxicity for fish"; and the OECD guideline No. 203: "Fish Acute Toxicity Test", Adopted 17 July, 1992.

The definitive test was performed with carp exposed to concentrations ranging from 1.0 to 10 mg/l in a static system. Acetone was used as a solvent in the stock solutions.

The 96h-LC50 for carp is 4.2 mg/l with 0 % mortality at 3.2 mg/l and 100 % mortality at 5.6 mg/l.

The BWZ of TK 12759, Araldite MY 0510 for acute toxicity in fish is 5.4.

PREFACE

Sponsor	Ciba-Geigy Ltd. Polymers Division R-1006.520 P.O. Box CH-4002 BASEL Switzerland
Study Monitor	Dr. E.G. Semadeni Additives Division R-1002.2.62
Testing Facility	NOTOX B.V. Hambakenwetering 3 5231 DD 's-Hertogenbosch The Netherlands
Study Director	Drs. M. Bogers
Study Plan	Start : 25 March, 1996 Completion : 19 April, 1996

TEST SUBSTANCE (NOTOX Substance Code: 59652)

Identification	TK 12759, Araldite MY 0510
Description	Clear yellow liquid (Determined at NOTOX)
Batch	50008M
Purity	> 95%
Test substance storage	In freezer in the dark
Stability under storage conditions	Stable
Expiry date	May 01, 1998
Stability in vehicle	Water: at least 96 h Acetone: at least 96 h

PURPOSE

The purpose of the study was to evaluate the test substance for its ability to generate acute toxic effects in Cyprinus carpio during an exposure period of 96 hours.

GUIDELINES

The study procedures described in this report were based on the following guidelines:

European Economic Community (EEC), EEC directive 92/69, Part C: Methods for the determination of ecotoxicity, Publication No. L383, December 1992, C.1. "Acute toxicity for fish".

The OECD guidelines for Testing of Chemicals, guideline No. 203: "Fish Acute Toxicity Test", Adopted 17 July, 1992.

ARCHIVING

NOTOX B.V. will archive the following data for at least 10 years: report, and raw data.

DEFINITIONS

Fish were considered to be dead when no reaction was observed after touching the caudal peduncle and visible breathing movements were absent.

The LC50 is the concentration killing 50% of the fish after a defined period of exposure.

TEST SPECIES

Species	Carp (Cyprinus carpio, Teleostei, Cyprinidae) (Linnaeus, 1758)
Source	Zodiac, proefacc, "De Haar Vissen", L.U. Wageningen, the Netherlands.
Mean length	Range-finding test: 1.90 ± 0.27 cm Final test: 1.96 ± 0.10 cm
Mean weight	Range-finding test: 0.30 ± 0.07 g Final test: 0.21 ± 0.03 cm
Characteristics	F1 from a single parent-pair bred in UV-treated water.
Reason for selection	This system has been selected as an internationally accepted species.
Total fish used	79

HOLDING

Quarantine/Acclimation	At least 12 days after delivery.
Medium	ISO-medium, formulated using Milli-Ro water (tap-water purified by reverse osmosis; Millipore Corp., Bedford, Mass., USA) with the following composition: Ca^{2+} 80 mg/l Mg^{2+} 12 mg/l Na^{+} 15 mg/l K^{+} 3 mg/l Cl^{-} 145 mg/l SO_4^{2-} 49 mg/l HCO_3^{-} 47 mg/l Hardness is 250 mg $CaCO_3$/l
Measurements	Oxygen concentration, pH, nitrate and nitrite concentration and ammonia concentration: once a week. Temperature: every day.
Feeding	Daily with Trouvit.
Control of sensitivity	A reference test with pentachlorophenol (PCP, SIGMA) is carried out within a period of 3 months. The results of this test are appended to the report.
Validity of batch	In the batch of fish used for the test, mortality during the seven days prior to the start of the test was less than 5%.

RANGE-FINDING TEST

A range-finding test was performed to provide information about the range of concentrations to be used in the final test:
three fish per concentration were exposed to a concentration range of 0.1 to 10 mg/l with an increasing factor of 10, while seven fish were exposed to 100 mg/l and the blank and solvent control.

FINAL STUDY: TEST PROCEDURE AND CONDITIONS

Test duration	96 hours
Test type	Static
Test vessels	5 litres, all-glass.
Test medium	ISO-medium, aerated until the dissolved oxygen concentration had reached saturation and the pH had stabilized. After aeration the hardness was 250 mg $CaCO_3$ per litre and the pH was 8.0 ± 0.2.
Number of fish	7 fish per concentration and controls.
Loading	0.74 g fish/litre, i.e. 7 fish per 2 litres of test medium.
Illumination	16 hours photoperiod daily
Aeration	The test media were aerated continuously.
Feeding	No feeding from 24 hours prior to the test and during the total test period.
Introduction of fish	Directly after preparation of the test media.
Euthanasia	At the end of the test the surviving fish were rapidly killed by exposing them to ca. 1.2% ethylene glycol monophenylether in water.

PREPARATION OF TEST SOLUTIONS

Stock solutions were prepared in acetone at 100, 56, 32, 18 and 10 mg/ml. A volume of 0.2 ml was taken from each stock and mixed with 2 litres of test medium providing the test concentrations of 10, 5.6, 3.2, 1.8 and 1.0 mg/l. The final test solutions were all clear without precipitation.

MEASUREMENTS AND RECORDINGS

Mortality and other effects	At (2), 24, 48, 72 and 96 hours following the start of exposure. Dead fish were removed when observed.
Dissolved oxygen content, pH and Temperature	Daily, beginning at the start of the test.

RESULTS

Range-finding test:

Incidence and total mortality:

Concentration (mg/l)	Initial number of fish	Cumulative mortality				Total Mortality (%)
		24 h	48 h	72 h	96 h	
Blank-control	7	0	0	0	0	0
Solvent-control	7	0	0	0	0	0
0.1	3	0	0	0	0	0
1	3	0	0	0	0	0
10	3	0	3	3	3	100
100	7	7	7	7	7	100

Definitive study:

Incidence and total mortality:

Concentration (mg/l)	Initial number of fish	Cumulative mortality				Total Mortality (%)
		24 h	48 h	72 h	96 h	
Blank-control	7	0	0	0	0	0
Solvent-control	7	0	0	0	0	0
1.0	7	0	0	0	0	0
1.8	7	0	0	0	0	0
3.2	7	0	0	0	0	0
5.6	7	0	3 *	7	7	100
10	7	0	7	7	7	100

* Surviving fish showed severe disturbance of swimming behaviour.

pH, dissolved oxygen and temperature:

Concentration (mg/l)	Day 0		Day 1		Day 2		Day 3		Day 4	
	pH	DO	pH	DO	pH	DO	pH	DO	pH	DO
Blank-control	8.1	9.1	8.0	9.0	7.8	8.8	7.9	8.9	7.9	8.9
Solvent-control	8.1	9.0	8.0	8.8	7.8	8.7	7.9	8.8	7.8	8.8
1.0	8.1	9.0	8.0	8.8	7.8	8.8	7.8	8.9	7.8	8.6
1.8	8.0	9.2	8.0	8.8	7.8	9.0	7.8	8.9	7.7	8.8
3.2	8.0	9.1	7.9	8.7	7.8	8.8	7.9	8.9	7.6	8.7
5.6	8.0	9.1	8.0	8.9	7.8	8.9	8.0	8.9		
10	8.0	9.2	8.0	8.8						
Temperature	20.5		20.4		20.5		20.5		20.4	

CONCLUSION

TK 12759, Araldite MY 0510:

The 96h-LC50 for carp is 4.2 mg/l with 0 % mortality at 3.2 mg/l and 100 % mortality at 5.6 mg/l.

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J. Weiss		✓
Ablage		

REPORT

PSEUDOMONAS CELL MULTIPLICATION INHIBITION TEST

WITH

TK 12759, ARALDITE MY 0510

NOTOX Project 169706
Ciba Project 964624
NOTOX Substance 59652

REPORT APPROVAL

STUDY DIRECTOR: Ir. Y.H.M. van Erp

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Date: May 22, 1996

MANAGEMENT: Ing. E.J. van de Waart
Section Head, Genetic &
Eco-Toxicology

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Date: 23/05/1996

SUMMARY

TK 12759, ARALDITE MY 0510 was investigated for its ability to inhibit the cell multiplication of the bacteria species Pseudomonas putida according to DIN 38412 Part L 8 (March 1991) and NEN-EN-ISO 10712 (1996).

Suspensions of Pseudomonas putida were exposed to concentrations ranging from 312.5 to 10,000 µg TK 12759, ARALDITE MY 0510 per litre. Acetone was used as vehicle.
After incubation the extinction values of the bacterial cell suspensions were measured. No toxicity was observed.

Based on the results of the test presently performed, the toxicity threshold value (EC10) of TK 12759, ARALDITE MY 0510 was over 10 mg/l. The "Bewertungszahl" (Assessment figure or value for bacterial toxicity) is therefore $\leq$ 4.9 (Bewertung wassergefährdender Stoffe, 1989).

The EC50 value for the reference substance, 3,5-dichlorophenol, tested during the same experiment was 20 mg/l. Since this was within the accepted range of 10-30 mg/l for the EC50 and the multiplication factor of the bacteria within the test was over 60, it was concluded that the test conditions were optimal and the results obtained were valid.

PREFACE

Sponsor	Ciba-Geigy Ltd. Polymers Division Bau R-1002.2.60 CH-4002 BASEL Switzerland
Study Monitor	Dr. E.G. Semadeni
Testing Facility	NOTOX B.V. Hambakenwetering 3 5231 DD 's-Hertogenbosch The Netherlands
Study Director	Ir. Y.H.M. van Erp
Technical Coordinator	A.M.C. Bertens
Study Plan	Start : March 28, 1996 Completed : March 29, 1996

TEST SUBSTANCE

Identification	TK 12759, Araldite MY 0510
Description	Clear yellow liquid (Determined at NOTOX)
Batch	50008M
Purity	> 95%
Test substance storage	In freezer in the dark
Stability under storage conditions	Stable
Expiry date	May 01, 1998
Stability in vehicle	Water : at least 96 h Acetone: at least 96 h

GUIDELINES

The study procedure described in this report was based on the following guidelines:

DIN 38412 Part L 8, March 1991: Determination of the Inhibition Effect of Water Constituents on Bacteria (Pseudomonas Cell Multiplication Inhibition Test) with minor modifications.

NEN-EN-ISO 10712, 1996: Water quality - Pseudomonas putida growth inhibition test (Pseudomonas cell multiplication inhibition test).

OBJECTIVE

Purpose of the study

The objective of this study is to evaluate the test substance for its ability to inhibit the cell multiplication of the bacterial species Pseudomonas putida. Furthermore, the toxicity threshold value (EC10) and EC50 value (if possible) of the test substance for Pseudomonas putida is determined.

Pseudomonas putida, an aerobic gram-negative bacterial species from the genus Pseudomonas, is a natural inhabitant of surface waters. This bacterium is a model water organism involved in biological self-purification. The inhibition of multiplication of bacterial cells of Pseudomonas putida is the selected parameter for dissolved toxic water ingredients.

DEFINITIONS

Effective concentration (EC10, EC50): the concentration of test substance determined after a test period of 16 ± 1 hour from the concentration-effect relationship at which cell multiplication is inhibited by 10 % or 50 % compared to that of the control. EC is expressed in mg test substance per litre.

Formazine nephelometric unit (FNU): Formazine turbidity units. The optical density of a bacterial suspension at λ=436 nm, measured as FNU.

Biomass: culture of bacterial cells after a certain time, expressed as FNU.

"Bewertungszahl": Assessment figure or value for bacterial toxicity expressed as the negative logarithmus of the EC10.
Example: EC10: 150 mg/l = 150 ppm = $1.5 \cdot 10^{-4}$ = Bewertungszahl 3.8.

TEST SYSTEM

Test system	Pseudomonas putida.
Strain	MIGULA, Berlin 33/2 (DSM 50026).
Source	BGA, Berlin, Germany.
Rationale	Recognized by international guidelines.

Stock cultures of the bacteria are stored in liquid nitrogen (-196°C).

TEST SUBSTANCE PREPARATION

Since the test substance was poorly soluble in water and no homogeneous suspension in water could be obtained, a stock solution (A) of 100 g per litre in acetone was prepared. Thorough mixing was performed to enhance the dissolving of the test substance. Solution A was diluted 1:1 with acetone (stock B, 50 g/l). In the same way stock C (25 g/l) to stock F (3.1 mg/l) were prepared.

REFERENCE SUBSTANCE

3,5 - Dichlorophenol (97 %, Aldrich).
A stock solution of 100 mg 3,5-dichlorophenol per litre in Milli-Q water was prepared.

EXPERIMENTAL CONDITIONS AND SOLUTIONS

Vessels	100 ml Erlenmeyer flasks
Incubation temperature	23 ± 1°C
Incubation time	
- preliminary suspension cultures	6 ± 1 hours
- exposure	16 ± 2 hours
Stock solution I	10.0 g $NaNO_3$ 2.4 g K_2HPO_4 1.2 g KH_2PO_4 1.0 g yeast extract Dissolved in 500 ml Milli-Q water.
Stock solution II	10.0 g $NaNO_3$ 2.4 g K_2HPO_4 1.2 g KH_2PO_4 Dissolved in 500 ml Milli-Q water.
Stock solution III	40.0 g D(+)- glucose Dissolved in 500 ml Milli-Q water.
Stock solution IV	0.01 g Fe (III) citrate 4.0 g $MgSO_4 . 7H_2O$ Dissolved in 1 l Milli-Q water.
Culture medium (stock)	Nutrient medium: 18 g of agar dissolved in water, plus 50 ml of solution I, 125 ml of solution III and 100 ml of solution IV, made up to one litre with water.

PERFORMANCE OF THE TEST

Preliminary suspension cultures

A small amount of bacteria from a maximally 7-day old stock culture of Pseudomonas putida was inoculated in pre-culture medium (25 ml of stock solution I and III plus 50 ml of solution IV, made up to one litre with water) in Erlenmeyer flasks. After incubation the final turbidity value of the bacterial suspension was adjusted up to FNU/436 nm = 50.

Exposure

Three parallel dilution series in 100 ml Erlenmeyer flasks covered with aluminium caps were prepared from the formulated test substance stock solution as described below.

Conc. test subst. (µg/l)	Milli-Q° water (ml)	Test solution[1] (µl of stock)		Stock solution II (ml)	III (ml)	IV (ml)	Bacterial suspension FNU/436=50 (ml)	total volume (ml)
10,000	32	4	A	1	1	2	4	40[2]
5,000	32	4	B	1	1	2	4	40
2,500	32	4	C	1	1	2	4	40
1,250	32	4	D	1	1	2	4	40
625	32	4	E	1	1	2	4	40
312	32	4	F	1	1	2	4	40
0[3]	32	4	acetone	1	1	2	4	40

° = Tap water purified by reverse osmosis and subsequently passed over activated carbon and ion-exchange cartridges (Millipore Corp., Bedford, Mass., USA).
[1] = Final volume of acetone was 0.01%.
[2] = pH of one of the test vessels was 8.1.
[3] = 10 control flasks were prepared.

Six flasks were made up identically as above, only 4 ml of pre-culture medium was added instead of the bacterial suspension (turbidity control).

The series of the reference substance were prepared by direct dilutions from the stock solution as follows:

Conc. reference subst. (mg/l)	Milli-Q water (ml)	Reference stock sol. (ml)	Stock solution II (ml)	III (ml)	IV (ml)	Bacterial suspension FNU/436=50 (ml)	total volume (ml)
40	16.0	16.0	1	1	2	4	40
28	20.8	11.2	1	1	2	4	40
20	24.0	8.0	1	1	2	4	40
14	26.4	5.6	1	1	2	4	40
10	28.0	4.0	1	1	2	4	40
7	29.2	2.8	1	1	2	4	40

After an appropriate time in an incubator shaker the turbidity values of the cell suspensions (biomasses) were measured.

MEASUREMENTS

Equipment	UV/VIS Spectrophotometer,Lambda 5 (Perkin Elmer, the Netherlands), λ = 436 nm; 10 mm layer
Calibration solution	Formazin standard suspension (in Formazine Turbidity Units) : FNU/436 nm = 100 FNU/436 nm = 75 FNU/436 nm = 50 FNU/436 nm = 25 FNU/436 nm = 12
Turbidity value bacterial suspension	FNU/436 nm = 50

CALCULATIONS

The percentage of cell multiplication inhibition for each tested concentration was calculated as follows:

$$I = \frac{Bc - Bn}{Bc - Bo} * 100$$

I Cell multiplication inhibition in %

Bn Measured biomass (extinction - extinction of turbidity control) at the end of the test period for the nth concentration of the test material

Bc Measured biomass (extinction) at the end of the test period in the control batch

Bo Measured biomass (extinction) at the start of the test (t=0) in the control batch.

The inhibition values calculated for each concentration were plotted against the corresponding concentration of the test substance.

ACCEPTABILITY OF THE TEST

The EC50 value of the reference substance 3,5-dichlorophenol should be in the accepted range of 10-30 mg/l.

The inoculum used in the control batch should be multiplied by a factor of at least 60 within the test period.

The preceding criteria are not absolute and other modifying factors might enter into the final evaluation decision.

RESULTS

Bacterial toxicity

After incubation extinction values were measured and the cell multiplication inhibition for each test concentration was determined (see Table 1).

Since no significant (significant: >10%) inhibition of cell multiplication was observed the toxicity threshold value of TK 12759, ARALDITE MY 0510 for Pseudomonas putida was over 10 mg/l.

Acceptability of the test

The EC50 value of 3,5-dichlorophenol determined for Pseudomonas putida as calculated by linear regression (Y = 6.8169 X -83.9824) was 20 mg/l (see Table 2).

The inoculum used in the control batch was multiplied by a factor 77 within the test period.

With respect to the accepted range of 10-30 mg/l for the EC50 of the reference substance and the multiplication factor of the bacteria within the test being over 60, it was concluded that the test conditions were optimal and the results obtained were valid.

CONCLUSION

Based on the results of the test presently performed, the toxicity threshold value (EC10) of TK 12759, ARALDITE MY 0510 was over 10,000 mg/l. The "Bewertungszahl" is therefore $\leq$ 4.9 (Bewertung wassergefährdender Stoffe, 1989).

TABLE 1

Extinction values of the inoculated dilution series I, II, III, the non-inoculated dilution series t (turbidity control) and the control flasks B.

CONCENTRATION of TK 12759, ARALDITE MY 0510 (µg/l)	EXTINCTION IN FNU 436 nm series I	II	III	MEAN I,II,II	t	CORRECTED MEAN	INHIBITION %
312.5	1.291	1.219	1.292	1.267	0.004	1.263	2.8
625	1.259	1.356	1.248	1.288	0.001	1.287	1.0
1250	1.231	1.201	1.173	1.202	0.001	1.201	7.7
2500	1.231	1.181	1.254	1.222	0.001	1.221	6.1
5000	1.291	1.394	1.294	1.326	0.001	1.325	-2.0
10000	1.172	1.283	1.222	1.226	0.002	1.224	5.9
CONTROL	1.320	1.175	1.348	1.201	1.349	1.251	1.410	1.376	1.297	1.265
MEAN	1.299									
BO	0.0168									

TABLE 2

Extinction values of the reference substance dilution series: 3,5-dichlorophenol.

CONCENTRATION 3,5-dichlorophenol (mg/l)	EXTINCTION IN FNU 436 nm series r	INHIBITION %
7	1.324	-1.9
10	1.264	2.7
14	1.269	2.4
20	0.424	68.2
28	0.015	100.1
40	0.016	100.0

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J. Weiss		✓
Ablage		

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ID# 11015

CIBA-GEIGY Limited
Basle, Switzerland

Exp. Toxicology Sissein
GU 2.1

ARALDITE 0510

REPORT ON

ACUTE ORAL LD50 IN THE RAT OF TX 12759

EPOXY 0510

Project No.:	800655
Sponsor:	Plastics and Additives Division
Test material received:	March 28, 1980
Additional identification:	Physical appearance: liquid Batch No. T-60011
The experiment started:	April 9, 1980
The experiment ended:	April 30, 1980
Study director:	Dr. med. vet. R. Bathe
Approved by:	Dr. med. vet. W. Gfeller
Reported:	May 20, 1980/wu
Archives:	Sissein MST 452

PERMANENT RECORD FILE
TOXICOLOGY

p. 1/9

800655

1. Summary and Conclusion

The acute oral LD50 of TK 12759 in rats of both sexes observed over a period of 14 days is 1037 (668-1403) mg/kg. The test material has therefore a slight acute toxicity to the rat by this route of administration.*

2. Method

a) Test material and formulation

TK 12759 was diluted to achieve the corresponding dosage level.

Volume (ml/kg body-weight): 10, 20

Vehicle: polyethylene glycol (PAG 400), Fluka AG, Buchs SG, Art. 81170

b) Animals

Healthy random bred rats of the Tif: RAIf (SPF) strain (7 to 8 weeks old) raised on the premises were used for these experiment. They were kept at a room temperature of 22 + 2°C, at a relative humidity of 55 + 10 % and on a 10 hours light cycle day. They received ad libitum rat food - NAFAG, Gossau SG - and water. Prior to treatment the animals were adapted to our laboratories for a minimum of 4 days. Bodyweights were recorded immediately prior to dosing (control weights) and at 7 and 14 days (Table 2).

During the treatment and observation period the animals were housed in groups of 5 in Macrolon cages (type 3), individually marked with picric acid.

---

* Qualification according to company standard.

c) Calculation

LD50 including 95 % confidence limits are calculated by the logit model.

d) Treatment and observations

Animals fasted overnight were treated by single oral intubation. Physical condition and rate of deaths were monitored throughout the whole observation period.

3. Results

a) Rate of deaths - Table 1

b) Bodyweight change - Table 2

c) Signs and symptoms - Tables 3 to 6

The surviving animals recovered within 9 to 12 days. They were submitted to a necropsy whenever they died, survivors at the end of the observation period.

d) Autopsies

Dead and killed Animals: No substance related gross organ changes were seen.

Table 1

RATE OF DEATHS

Dose mg/kg	Sex	Total Number animals in group	Total Number animals dead	Death rate percentage	Time of death after dosing: Hours after dosing 1	2	3	5	24	Days after dosing 2	3	4	5	6	7	8	9	10	11	12	13	14
400	♂	5	0	0																		
1000		5	2	40					2													
2000		5	4	80					1	1		1		1								
3000		5	5	100			2	1	2													
400	♀	5	0	0																		
1000		5	4	80					3					1								
2000		5	4	80					1		1	1		1								
3000		5	5	100		1		3	1													

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Exp. Toxicology Sisseln
GU 2.1

Table 2 - BODYWEIGHT CHANGE

		Dose (mg/kg)						
		400	1000	2000	3000			
Day 1 ♂	MEAN BODYWEIGHT/SD (g)	176/4,2	201/17,6	205/14,8	171/2,5			
Day 1 ♀		175/5,3	178/9,0	179/5,7	169/5,5			
Day 7 ♂		219/2,9	163/15,9	170	—			
Day 7 ♀		194/5,0	156	130	—			
Day 14 ♂		263/6,8	228/23,9	211	—			
Day 14 ♀		221/9,9	197	135	—			

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Exp. Toxicology Sisseln
GU 2.1

Table 3 - Signs and symptoms

Dose (mg/kg): 400

Signs and symptoms	Hours					Days												
	1	2	3	5	24	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.
Sedation																		
Dyspnoea	+	+	+	+	+	+	+	+	+	+								
Dacryorrhoea																		
Chromodacryorrhoea																		
Minorrhoea																		
Epistaxis																		
Exophthalmos					+	+	+	+										
Salivation																		
Ruffled fur	+	+	+	+	+	+	+	+	+	+	+	+						
Pallor																		
Cyanosis																		
Diarrhoea																		
Body position (ventral)																		
Body position (lateral)																		
Body position (curved)	+	+	+	+	+	+	+	+	+	+								
Ataxia																		
Trismus																		
Tremor																		
Tonic clonic muscle spasms																		
Convulsions																		

Key: + = slight, ++ = moderate, +++ = severe

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Exp. Toxicology Sisseln
GU 2.1

Table 4 - Signs and symptoms

Dose (mg/kg): 1000

Signs and symptoms	Hours					Days												
	1	2	3	5	24	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.
Sedation		+	+	+														
Dyspnoea	+	+	+	+	+	+	+	+	+	+								
Dacryorrhoea																		
Chromodacryorrhoea																		
Rhinorrhoea																		
Epistaxis																		
Exophthalmos	+	+	+	+	+	+	+	+	+	+	+							
Salivation																		
Ruffled fur	+	+	++	++	++	++	++	++	++	++	+	+						
Pallor																		
Cyanosis																		
Diarrhoea					+	+	+											
Body position (ventral)																		
Body position (lateral)																		
Body position (curved)	+	+	+	+	+	+	+	+	+	+								
Ataxia																		
Trismus																		
Tremor																		
Tonic clonic muscle spasms																		
Convulsions																		

Key: + = slight, ++ = moderate, +++ = severe

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Exp. Toxicology *Sisseln*
GU 2.1

Table 5 - Signs and symptoms

Dose (mg/kg): 2000

Signs and symptoms	Hours					Days												
	1	2	3	5	24	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.
Sedation		+	+	+	+	+	+	+	+	+								
Dysphoea	+	+	+	+	+	+	+	++	++	++	+	+	+	+				
Dacryorrhoea																		
Chromodacryorrhoea																		
Rhinorrhoea																		
Epistaxis																		
Exophthalmos	+	+	+	+	+	+	+	+	+	+	+							
Salivation																		
Ruffled fur	+	++	++	++	++	++	++	++	++	++	++	+	+	+	+			
Pallor																		
Cyanosis																		
Diarrhoea					+	+	+	+	+	+								
Body position (ventral)																		
Body position (lateral)																		
Body position (curved)	+	+	+	+	+	+	+	+	+	+	+	+	+	+				
Ataxia																		
Trismus																		
Tremor																		
Tonic clonic muscle spasms																		
Convulsions																		

Key: + = slight, ++ = moderate, +++ = severe

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Exp. Toxicology Sisseln
GU 2.1

Table 6 - Signs and symptoms

Dose (mg/kg): 3000

Signs and symptoms	Hours					Days												
	1	2	3	5	24	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.
Sedation	+	+	+	+														
Dyspnoea	+	+	+	+														
Dacryorrhoea																		
Chromodacryorrhoea																		
Rhinorrhoea																		
Epistaxis																		
Exophthalmos	+	+	+	+														
Salivation																		
Ruffled fur	+	+	+	+														
Pallor																		
Cyanosis																		
Diarrhoea	+	+	+	+														
Body position (ventral)																		
Body position (lateral)																		
Body position (curved)	+	+	+	+														
Ataxia																		
Trismus																		
Tremor																		
Tonic clonic muscle spasms																		
Convulsions																		

Key: + = slight, ++ = moderate, +++ = severe

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CD 5/103/80

ID #11016

CIBA-GEIGY Limited
Basle, Switzerland

Exp. Toxicology Sisseln
GU 2.1

RESINS
TOXICITY

EPOXY 0510

REPORT ON

ACUTE ORAL LD50 IN THE MOUSE OF

TK 12759 ARALDITE 0510

Project No.:	800654
Sponsor:	Plastics and Additives Division
Test material received:	March 28, 1980
Additional identification:	Physical appearance: liquid Batch No.: T-60011
The experiment started:	April 15, 1980
The experiment ended:	September 23, 1980

G. Sarasin

Study director: Dr. phil. II G. Sarasin

Reported: October 20, 1980/wu

Archives: Sisseln WST 452

PERMANENT RECORD FILE
TOXICOLOGY

800654

1. Summary and Conclusion

The acute oral LD50 of TK 12759 in mice of both sexes observed over a period of 14 days is 1413 (1024-1873) mg/kg. The test material has therefore a slight acute toxicity to the mouse by this route of administration.*

2. Method

a) Test material and formulation

TK 12759 was diluted to achieve the corresponding dosage level.

Volume (ml/kg body-weight):	10, 20
Vehicle:	polyethylene glycol (PAG 400), Fluka AG, Buchs SG, Art. 81170

b) Animals

Healthy random bred mice of the Tif: MAG (SPF) strain (4 to 5 weeks old) raised on the premises were used for this experiment. They were kept at a room temperature of 22 ± 2°C, at a relative humidity of 55 ± 10 % and on a 10 hours light cycle day. They received ad libitum mouse food - NAFAG, Gossau SG - and water. Prior to treatment the animals were adapted to our laboratories for a minimum of 4 days. Bodyweights were recorded immediately prior to dosing (control weights) and at 7 and 14 days (Table 2).

During the treatment and observation period the animals were housed in groups of 5 in Macrolon cages (type 2), individually marked with picric acid.

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* Qualification according to company standard.

c) Calculation

LD50 including 95 % confidence limits are calculated by the logit model.

d) Treatment and observations

Animals fasted overnight were treated by single oral intubation. Physical condition and rate of deaths were monitored throughout the whole observation period.

3. Results

a) Rate of deaths - Table 1

b) Bodyweight change - Table 2

c) Signs and symptoms - Tables 3 to 7

The surviving animals recovered within 9 days. They were submitted to a necropsy whenever they died, survivors at the end of the observation period.

d) Autopsies

Dead and killed Animals: No substance related gross organ changes were seen.

Table 1

RATE OF DEATHS

Dose mg/kg	Sex	Total Number animals in group	Total Number animals dead	Death rate percentage	Number of Animals died within: Hours after Treatment 1	2	3	5	24	Days after Treatment 2	3	4	5	6	7	8	9	10	11	12	13	14	.
600	♂	5	0	0																			
1000		5	1	20							1												
1500		5	4	80				1	3														
2500		5	4	80				1	1		2												
3000		5	4	80		1	3																
*																							
600	♀	5	1	20								1											
1000		5	0	0																			
1500		5	3	60	1			1	1														
2500		5	4	80				2	2														
3000		5	5	100		1	4																

* No higher doses were possible

800654

Exp. Toxicology Sisseln
GU 2.1

Table 2 - BODYWEIGHT CHANGE

		Dose (mg/kg)						
		600	1000	1500	2500	3000		
Day 1 ♂	MEAN BODYWEIGHT/SD (g)	18/1.2	24/3.7	26/2.3	26/1.1	24/1.1		
Day 1 ♀		19/0.9	23/0.8	22/1.1	22/2.3	20/1.7		
Day 7 ♂		25/1.7	27/7.2	26	24	27		
Day 7 ♀		23/0.9	28/1.9	19/2.1	22	—		
Day 14 ♂		30/2.3	30/8.5	30	26	31		
Day 14 ♀		25/0.9	28/1.1	21/2.1	27	—		

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Exp. Toxicology Sisseln
GU 2.1

Table 3 - Signs and symptoms

Dose (mg/kg): 600

Signs and symptoms	Hours					Days												
	1	2	3	5	24	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.
Sedation		+	+	+	+	+	+											
Dyspnoea	+	+	+	+	+	+	+	+	+	+	+							
Dacryorrhoea																		
Chromodacryorrhoea																		
Rinorrhoea																		
Epistaxis																		
Exophthalmos																		
Salivation																		
Ruffled fur	+	+	+	+	+	+	+	+	+	+	+	+						
Pallor																		
Cyanosis																		
Diarrhoea																		
Body position (ventral)																		
Body position (lateral)																		
Body position (curved)	+	+	+	+	+	+	+	+	+	+	+							
Ataxia																		
Trismus																		
Tremor																		
Tonic clonic muscle spasms																		
Convulsions																		

Key: + = slight, ++ = moderate, +++ = severe

800654

Exp. Toxicology Sisseln
GU 2.1

Table 4 - Signs and symptoms

Dose (mg/kg): 1000

Signs and symptoms	Hours					Days												
	1	2	3	5	24	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.
Sedation		+	+	+	+	+	+	+	+	+	+	+						
Dyspnoea	+	+	+	+	+	+	+	+	+	+	+							
Dacryorrhoea																		
Chromodacryorrhoea																		
Rhinorrhoea																		
Epistaxis																		
Exophthalmos																		
Salivation																		
Ruffled fur	+	+	+	+	+	+	+	+	+	+	+	+						
Pallor																		
Cyanosis																		
Diarrhoea																		
Body position (ventral)																		
Body position (lateral)																		
Body position (curved)	+	+	+	+	+	+	+	+	+	+	+							
Ataxia																		
Trismus																		
Tremor																		
Tonic clonic muscle spasms																		
Convulsions																		

Key: + = slight, ++ = moderate, +++ = severe

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Exp. Toxicology Sisseln
GU 2.1

Table 5 - Signs and symptoms

Dose (mg/kg): 1500

Signs and symptoms	Hours					Days												
	1	2	3	5	24	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.
Sedation	+/++	+/++	++	+/++	+	+/-	+	+	+									
Dyspnoea	+	+	+/-	+	+	+	+	+/-	+	+	+/-							
Dacryorrhoea																		
Chromodacryorrhoea																		
Rinorrhoea																		
Epistaxis																		
Exophthalmos																		
Salivation																		
Ruffled fur	++	++	++	++	+/++	++	+	+	+	+	+	+						
Pallor																		
Cyanosis																		
Diarrhoea																		
Body position (ventral)																		
Body position (lateral)																		
Body position (curved)	+	+	+	+	+	+	+	+	+	+	+							
Ataxia																		
Trismus																		
Tremor																		
Tonic clonic muscle spasms																		
Convulsions																		

Key: + = slight, +- = moderate, +++ = severe

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Exp. Toxicology Sisseln
GU 2.1

Table 6 - Signs and symptoms

Dose (mg/kg): 2500

Signs and symptoms	Hours					Days												
	1	2	3	5	24	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.
Sedation	++	++	++	++	+	+	+	+	+									
Dyspnoea	+	+	+	+	+	+	+	+	+	+	+							
Dacryorrhoea																		
Chromodacryorrhoea																		
Rinorrhoea																		
Epistaxis																		
Exophthalmos																		
Salivation																		
Ruffled fur	+	+	+	+	+	+	+	+	+	+	+	+						
Pallor																		
Cyanosis																		
Diarrhoea																		
Body position (ventral)	+	+	+	+														
Body position (lateral)																		
Body position (curved)	+	+	+	+	+	+	+	+	+	+	+							
Ataxia																		
Trismus																		
Tremor																		
Tonic clonic muscle spasms																		
Convulsions																		

Key: + = slight, ++ = moderate, +++ = severe

800654

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Exp. Toxicology Sisseln
GU 2.1

Table 7 - *Signs and symptoms*

Dose (mg/kg): 3000

Signs and symptoms	Hours					Days												
	1	2	3	5	24	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.
Sedation	+++	++	++	++	++	+												
Dyspnoea	++	+	+	+	+	+	+	+	+	+	+							
Dacryorrhoea																		
Chromodacryorrhoea																		
Rhinorrhoea																		
Epistaxis																		
Exophthalmos																		
Salivation																		
Ruffled fur	+	+	+	+	+	+	+	+	+	+	+	+						
Pallor																		
Cyanosis																		
Diarrhoea																		
Body position (ventral)	+																	
Body position (lateral)	+																	
Body position (curved)	+	+	+	+	+	+	+	+	+	+								
Ataxia																		
Trismus																		
Tremor																		
Tonic clonic muscle spasms																		
Convulsions	+																	

Key: + = slight, ++ = moderate, +++ = severe

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CO 5/103/76

ID#11014

CIBA-GEIGY Limited
Basle, Switzerland

Exp. Toxicology Sisseln
GU 2.1

RESIN

TOXICITY

REPORT ON
ARALDITE 0510
ACUTE ORAL LD50 IN THE CHINESE HAMSTER OF TK 12759

EPOXY 0510

Project No.:	800656
Sponsor:	Plastics and Additives Division
Test material received:	March 28, 1980
Additional identification:	Physical appearance: liquid Batch No.: T-60011
The experiment started:	May 1, 1980
The experiment ended:	July 7, 1980
Study director:	Bathe Dr. med. vet. R. Bathe
Approved by:	W. Gfeller Dr. med. vet. W. Gfeller
Reported:	August 11, 1980/wu
Archives:	Sisseln HST 452

PERMANENT RECORD FILE
TOXICOLOGY

800656

1. Summary and Conclusion

The acute oral LD50 of TK 12759 in Chinese hamsters of both sexes observed over a period of 14 days is 2739 (2276-3429) mg/kg. The test material has therefore a slight acute toxicity to the Chinese hamster by this route of administration.*

2. Method

a) Test material and formulation

TK 12759 was diluted to achieve the corresponding dosage level.

Volume (ml/kg body-weight): 10, 20

Vehicle: polyethylene glycol (PAG 400), Fluka AG, Buchs SG, Art. 81170

b) Animals

Healthy random bred Chinese hamsters (9 to 11 weeks old) raised on the premises were used for these experiment. They were kept at a room temperature of 22 ± 2°C, at a relative humidity of 55 ± 10 % and on a 10 hours light cycle day. They received ad libitum hamster food - NAFAG, Gossau SG - and water. Prior to treatment the animals were adapted to our laboratories for a minimum of 4 days. Bodyweights were recorded immediately prior to dosing (control weights) and at 7 and 14 days (Table 2).

During the treatment and observation period the animals were housed in groups of 5 in Macrolon cages (type 3), individually marked with picric acid.

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* Qualification according to company standard.

c) Calculation

LD50 including 95 % confidence limits are calculated by the logit model.

d) Treatment and observations

Animals fasted overnight were treated by single oral intubation. Physical condition and rate of deaths were monitored throughout the whole observation period.

3. Results

a) Rate of deaths - Table 1

b) Bodyweight change - Table 2

c) Signs and symptoms - Tables 3 to 7

The surviving animals recovered within 7 to 28 days. They were submitted to a necropsy whenever they died, survivors at the end of the observation period.

d) Autopsies

Dead and killed Animals: No substance related gross organ changes were seen

Table 1

RATE OF DEATHS

Dose mg/kg	Sex	Total Number animals in group	Total Number animals dead	Death rate percentage	Number of Animals died within																		
					Hours after Treatment					Days after Treatment													
					1	2	3	5	24	2	3	4	5	6	7	8	9	10	11	12	13	14	18
1000	♂	5	0	0																			
2000		5	0	0																			
2500		5	2	40									1										1
3000		5	3	60							1	1	1										
5000		5	5	100					1	2	2												
1000	♀	5	0	0																			
2000		5	0	0																			
2500		5	0	0																			
3000		5	5	100					1			3			1								
5000		5	5	100							1	2	1					1					

Exp. Toxicology Sisseln
GU 2.1

Table 2 - BODYWEIGHT CHANGE

		Dose (mg/kg)						
		1000	2000	2500	3000	5000		
Day 1 ♂	MEAN BODYWEIGHT/SD (g)	31/1.0	30/1.3	28/3.2	29/2.8	29/1.9		
Day 1 ♀		30/2.0	28/2.3	27/2.0	27/0.5	30/1.8		
Day 7 ♂		31/4.5	31/2.8	26/3.5	20/3.5	—		
Day 7 ♀		26/2.7	29/1.6	26/2.6	—	20		
Day 14 ♂		30/1.7	28/1.9	23/3.2	22/0.0	—		
Day 14 ♀		26/2.2	26/2.2	25/1.5	—	—		

Exp. Toxicology Sisseln
GU 2.1

Table 3 - Signs and symptoms

Dose (mg/kg): 1000

Signs and symptoms	Hours					Days												
	1	2	3	5	24	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.
Sedation	+	+	+	+	+	+												
Dyspnoea	+	+	+	+	+	+	+	+	+	+								
Dacryorrhoea																		
Chromodacryorrhoea																		
Rinorrhoea																		
Epistaxis																		
Exophthalmos	+	+	+	+	+	+	+											
Salivation																		
Ruffled fur	+	+	+	+	+	+	+	+	+	+	+	+						
Pallor																		
Cyanosis																		
Diarrhoea																		
Body position (ventral)																		
Body position (lateral)																		
Body position (curved)	+	+	+	+	+	+	+	+	+	+								
Ataxia																		
Trismus																		
Tremor																		
Tonic clonic muscle spasms																		
Convulsions																		

Key: + = slight, ++ = moderate, +++ = severe

Exp. Toxicology Sisseln
GU 2.1

Table 4 - Signs and symptoms

Dose (mg/kg): 2000

Signs and symptoms	Hours					Days													
	1	2	3	5	24	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.
Sedation	+	+	+	+	+														
Dyspnoea	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
Dacryorrhoea																			
Chromodacryorrhoea																			
Rhinorrhoea																			
Epistaxis																			
Exophthalmos	+	+	+	+	+	+	+	+	+	+	+								
Salivation																			
Ruffled fur	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
Pallor																			
Cyanosis																			
Diarrhoea																			
Body position (ventral)																			
Body position (lateral)																			
Body position (curved)	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
Ataxia																			
Trismus																			
Tremor																			
Tonic clonic muscle spasms																			
Convulsions																			

Key: + = slight, ++ = moderate, +++ = severe

Exp. Toxicology Sissein
GU 2.1

Table 5 - Signs and symptoms

Dose (mg/kg): 2500

Signs and symptoms	Hours					Days												
	1	2	3	5	24	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.
Sedation	+	+	+	+	+	+	+	+										
Dyspnoea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Dacryorrhoea																		
Chromodacryorrhoea																		
Rinorrhoea																		
Epistaxis																		
Exophthalmos	+	+	+	+	+	+	+	+	+	+	+							
Salivation																		
Ruffled fur	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pallor																		
Cyanosis																		
Diarrhoea																		
Body position (ventral)																		
Body position (lateral)																		
Body position (curved)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ataxia																		
Trismus																		
Tremor																		
Tonic clonic muscle spasms																		
Convulsions																		

Key: + = slight, +- = moderate, +-+ = severe

Exp. Toxicology Sisseln
GU 2.1

Table 6 - Signs and symptoms

Dose (mg/kg): 3000

Signs and symptoms	Hours					Days												
	1	2	3	5	24	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.
Sedation	+	+	+	+	+	+	+	+	+	+	+							
Dyspnoea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Dacryorrhoea																		
Chromodacryorrhoea																		
Rinorrhoea																		
Epistaxis																		
Exophthalmos	+	+	+	+	+	+	+	+	+	+	+							
Salivation																		
Ruffled fur	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+	+	+
Pallor																		
Cyanosis																		
Diarrhoea																		
Body position (ventral)																		
Body position (lateral)																		
Body position (curved)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ataxia																		
Trismus																		
Tremor																		
Tonic clonic muscle spasms																		
Convulsions																		

Key: + = slight, ++ = moderate, +++ = severe

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Exp. Toxicology Sisseln
GU 2.1

Table 7 - Signs and symptoms

Dose (mg/kg): 5000

Signs and symptoms	Hours					Days												
	1	2	3	5	24	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.
Sedation	+	+	+	+	+	+	+	+	+	+	+	+	+					
Dyspnoea	+	+	+	+	+	+	+	+	+	+	+	+	+					
Dacryorrhoea																		
Chromodacryorrhoea																		
Rinorrhoea																		
Epistaxis																		
Exophthalmos	+	+	+	+	+	+	+	+	+	+								
Salivation																		
Ruffled fur	++	++	++	++	++	++	++	++	++	++	++	++	++					
Pallor																		
Cyanosis																		
Diarrhoea																		
Body position (ventral)																		
Body position (lateral)																		
Body position (curved)	+	+	+	+	+	+	+	+	+	+	+	+	+					
Ataxia																		
Trismus																		
Tremor																		
Tonic clonic muscle spasms																		
Convulsions																		

Key: + = slight, ++ = moderate, +++ = severe

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CD 5/106/7

I.D. # 09810

Industrial BIO-TEST Laboratories, Inc.
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

Ciba-Geigy Epoxy 0500

REPORT TO

CIBA-GEIGY CORPORATION

ACUTE ORAL TOXICITY STUDY WITH EPOXY 0500 IN MALE ALBINO RATS

DECEMBER 17, 1975

IBT NO. 601-07231

Industrial BIO-TEST Laboratories, Inc.

REPORT TO

CIBA-GEIGY CORPORATION

Acute Oral Toxicity Study - Male Albino Rats

Results

IBT No.: 601-07231
Test Material: Epoxy 0500
Physical Description: Brown liquid
Form Administered: As suspensions in propylene glycol; 1.0 percent (w/v) at 30 and 100 mg/kg, 10.0 percent (w/v) at 300 and 1,000 mg/kg and undiluted at 3,000 and 10,000 mg/kg

Classification: Slightly Toxic
Approximate Acute Oral
Lethal Dose > 1,000 < 3,000 mg/kg

I. Mortality and Body Weights

Dose Level (mg/kg)	Animal Number	Individual Body Weights (grams) Test Day Number: 0	14	Number Dead / Number Tested	Percent Dead
30	1	186	322	0/1	0
100	2	172	276	0/1	0
300	3	218	306	0/1	0
1,000	4	184	270	0/1	0
3,000	5	184	(5 days)	1/1	100
10,000	6	186	(6-22 hours)	1/1	100

Note: Figures in parentheses indicate time of death.

II. Reactions and Pathology

Hypoactivity and ruffed fur were noted in all the animals within 5 to 15 minutes post-oral administration of Epoxy 0500 and diarrhea was noted in the animals at the 3,000 and 10,000 mg/kg dose levels within 2 hours. Within 6-22 hours, all the animals at the 30 through 1,000 mg/kg dose levels appeared normal.

Necropsy examination of the animals that died revealed severe gastroenteritis. Examination of the survivors of the 14-day investigation revealed red, mottled lungs in animal No. 4. No other gross pathologic alterations were noted.

Respectfully submitted,

INDUSTRIAL BIO-TEST LABORATORIES, INC.

Prepared by: Wendy A. Harrison
Wendy A. Harrison, B.S.
Technician
Acute Toxicity

Approved by: C. W. Mastri
C. W. Mastri, B.S.
Section Head, Acute Toxicity

chm

CD 5/106/11

/ Im 146

I.D. # 09813

Industrial Medicine

SEP 29 1975

Industrial BIO-TEST *Laboratories, Inc.*
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

Ciba-Geigy Epoxy 0500

REPORT TO

CIBA-GEIGY CORPORATION

ACUTE VAPOR INHALATION TOXICITY STUDY WITH EPOXY 0500 IN ALBINO RATS

SEPTEMBER 25, 1975

IBT NO. 663-07230

Batch No. N-68

Industrial **BIO-TEST** *Laboratories, Inc.*
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

September 25, 1975

Dr. Edgar M. Flint
Associate Director
Industrial Medicine
CIBA-GEIGY Corporation
Ardsley, New York 10502

Dear Dr. Flint:

Re: IBT No. 663-07230 - Acute Vapor Inhalation
Toxicity Study with Epoxy 0500 in Albino Rats

We are submitting herewith our laboratory report prepared in connection with the above study.

Very truly yours,

J. C. Calandra

J. C. Calandra
President

JCC:bp

Industrial BIO-TEST Laboratories, Inc.

REPORT TO CIBA-GEIGY CORPORATION

ACUTE VAPOR INHALATION TOXICITY STUDY IN RATS

Test Material: Epoxy 0500, Batch No. N-68, 6-12-75.
Form Administered: Vapor
Acute LC_{50}: > 46.2 mg/m^3 air

Strain: Charles River Rats
Exposure Time: 4 hours
Observation Period: 14 days
IBT No. 663-07230

Generation of Material Exposure:

Vapors were generated by passing a stream of clean, dry air (-40°C dewpoint) through the undiluted test material contained in a gas washing bottle.

Chamber Conditions Group No.	Size (liters)	Atmospheric Pressure (inches Hg)	Temperature (°C)	Air Flow (l/min)
I	80	30.06	24	3.6

Results Group No.	Total Number of Animals Male/Female	Nominal Concentration	Mortality Male-Female	Weight Gain Male-Female (grams)
I	5/5	46.2 mg/m^3air*	0/5 - 0/5	113/40

*Maximum attainable concentration at conditions stated.

Remarks

The untoward behavioral reaction observed during the exposure was hypoactivity. The average 2-week body weight gains were within the normal limits. Necropsy, performed on all rats at the end of the observation period, did not reveal any gross pathologic alterations.

Respectfully submitted,

INDUSTRIAL BIO-TEST LABORATORIES, INC.

Prepared by: Larry L. Horath — Approved by: John W. Goode

Larry L. Horath, B.S.
Assistant Toxicologist
Inhalation Toxicity

John W. Goode, Ph.D.
Manager
Decatur Research Laboratories

mk

Industrial BIO-TEST Laboratories, Inc.

PROCEDURE FOR ACUTE VAPOR INHALATION TOXICITY STUDY

Young adult albino rats were employed as test animals. The rats were selected after having been under observation for at least 5 days to insure their general health and suitability for testing. The animals were housed in stainless steel cages and permitted a standard laboratory diet* plus water ad libitum, except during inhalation exposure.

During the exposure period, observations were made with respect to incidence of mortality and reactions displayed. At the end of the exposure period, the rats were returned to their cages for observation.

A body weight was determined for each animal prior to inhalation exposure and for each surviving animal at the end of the observation period. The data were recorded as an index to growth.

Gross pathologic examinations were scheduled to be conducted upon all animals which might succumb during the test period and upon those sacrificed at the end of the observation period.

Test animals were exposed in a specially constructed inhalation chamber. The chamber was designed so that the animals could be introduced into the test atmosphere after 99 percent of the maximum vapor concentration was established. Each animal was caged separately during exposure to minimize dilution of inspired air by animal fur.

Vapor was generated by bubbling a stream of clean, dry air (-40°C dewpoint) through the undiluted test material. The resulting air-vapor mixture was introduced into the exposure chamber at the top center, dispersed by a baffle plate and exhausted at the bottom of the chamber. The vapor generator was of all-glass construction and was linked to the exposure chamber by a short length of Tygon tubing. The air flow rate through the system was measured with a rotameter connected upstream of the generator. The rotameter was calibrated with a wet-test meter after the exposure was completed. The average nominal vapor concentration was calculated by dividing the generator weight loss by the total volume of air used during the test.

Whenever possible, the LC_{50} was calculated using the method of Litchfield and Wilcoxon**.

* Wayne LAB-BLOX for Rats, Allied Mills, Inc., Chicago, Illinois.

** Litchfield, J. T., Jr. and Wilcoxon, F. "A Simplified Method of Evaluating Dose-Effect Experiments," J. Pharm. & Exp. Ther. 96 99 (1949)

[illegible] EPOXY 0500 9/29/75

Acute Vapor Inhalation - Rat

Log Date 9/25/75

Test Description/Species

Batch No. N-68

Report Date IBT # 663-07230

IW# 146 Lab.7#

10/9/75 Coded 2/5/85 10/6/75

☒ BK Distribution: H.W. Zussman FEL

☐ ZN K.T. Shen D. Gordon EMF

☒ LG F. Pechorr CB

[15]

PERMANENT RECORD FILE

Industrial Medicine

CO 5/103/82

ID# 11017

CIBA-GEIGY Limited
Basle, Switzerland

Exp. Toxicology Sisseln
GU 2.1

EPA/OTS 0510

REPORT ON

ACUTE DERMAL LD50 IN THE RAT OF TK 12759

EPOXY 05.10

Project No.:	800659
Sponsor:	Plastics and Additives Division
Test material received:	March 28, 1980
Additional identification:	Physical appearance: liquid Batch No.: T-60011
The experiment started:	May 21, 1980
The experiment ended:	June 10, 1980
Study director:	Dr. med. vet. R. Bathe
Approved by:	Dr. med. vet. W. Gfeller
Reported:	June 25, 1980/wu
Archives:	Sisseln HST 452

PERMANENT RECORD FILE
TOXICOLOGY

800659

1. Summary and Conclusion

The acute dermal LD50 of TK 12759 in rats of both sexes observed over a period of 14 days is greater than 4000 mg/kg. The test material has therefore practically no acute toxicity to the rat by this route of application.*

Skin irritation did occur.

2. Method

a) Test material and formulation

TK 12759 was applied as a concentrate to achieve the corresponding dosage level.

Volume (ml/kg body-weight): 3 - 4

Vehicle: conc.

b) Animals

Healthy random bred rats of the Tif: RAIf (SPF) strain (8 to 9 weeks old) raised on the premises were used for these experiment. They were kept at a room temperature of 22 ± 2°C, at a relative humidity of 55 ± 10 % and on a 10 hours light cycle day. They received ad libitum rat food - NAFAG, Gossau SG - and water. Prior to treatment the animals were adapted to our laboratories for a minimum of 4 days. Bodyweights were recorded immediately prior to dosing (control weights) and at 7 and 14 days (Table 2).

---

* Qualification according to company standard.

c) Calculation

LD50 including 95 % confidence limits are calculated by the logit model.

d) Treatment* and observations

During the treatment and observation period the rats were housed individually in Macrolon cages (type 2). Approximately 24 hours before treatment an area on the back of the rats of approximately 60 square cm was shaved with an electric clipper. For treatment the test material was evenly dispersed on the skin with a syringe and was covered with an occlusive dressing which was fastened around the trunk with an adhesive elastic bandage. After 24 hours the dressing was removed, the skin was cleaned with lukewarm water and the reaction of the skin was appraised.

3. Results

a) Rate of deaths - Table 1

b) Bodyweight change - Table 2

c) Signs and symptoms - Tables 3 to 5

The animals recovered from systemic symptoms within 9 days. They were submitted to a necropsy at the end of the observation period.

d) Autopsies

Killed Animals: No substance related gross organ changes were seen.

---

* Noakes, D.N. and Sanderson, D.M.
A method for determining the dermal toxicity of pesticides.
Brit. J. Industr. Med., 26, 59-64, 1969

Table 1

RATE OF DEATHS

Dose mg/kg	Sex	Total Number animals in group	Total Number animals dead	Death rate percentage	Time of death after dosing																		
					Hours after dosing					Days after dosing													
					1	2	3	5	24	2	3	4	5	6	7	8	9	10	11	12	13	14	.
control	♂	5	0	0																			
3000		5	0	0																			
3500		5	0	0																			
4000		5	0	0																			
*																							
control	♀	5	0	0																			
3000		5	0	0																			
3500		5	0	0																			
4000		5	0	0																			
*																							

* No higher doses were possible

Exp. Toxicology Sisseln
GU 2.1

Table 2 - BODYWEIGHT CHANGE

		Dose (mg/kg)						
		control	3000	3500	4000			
Day 1 ♂	MEAN BODYWEIGHT/SD (g)	211/9.7	238/9.6	222/9.5	230/11.8			
Day 1 ♀		191/9.7	187/6.0	193/4.1	191/9.4			
Day 7 ♂		237/1.2	248/25.4	233/15.0	228/15.3			
Day 7 ♀		196/15.3	185/8.0	192/13.6	183/11.8			
Day 14 ♂		282/22.2	270/39.3	282/25.2	276/18.4			
Day 14 ♀		210/18.6	209/12.6	211/15.2	203/10.0			

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Exp. Toxicology Sisseln
GU 2.1

TABLE 3 - SIGNS AND SYMPTOMS

Dose (mg/kg): 3000

Signs and symptoms		HOURS					DAYS												
		1	2	3	5	24	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.
Sedation		+	+	+	+	+	+												
Dyspnoea		+	+	+	+	+	+	+	+	+	+								
Dacryorrhoea																			
Chromodacryorrhoea																			
Rinorrhoea																			
Epistaxis																			
Exophthalmos		+	+	+	+	+	+	+	+	+	+	+							
Salivation																			
Ruffled fur		+	+	+	+	+	+	+	+	+	+	+	+						
Pallor																			
Cyanosis																			
Diarrhoea																			
Body position (ventral)																			
Body position (lateral)																			
Body position (curved)		+	+	+	+	+	+	+	+	+	+								
Ataxia																			
Trismus																			
Tremor																			
Tonic clonic muscle spasms																			
Convulsions																			
SKIN IRRITATION	erythema						+ +	+ +	+ +	+ +	+ +	+ +	+	+					
	oedema																		
	necrosis																		

[illegible]

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GU 2.1

TABLE 4 - SIGNS AND SYMPTOMS

Dose (mg/kg): 3500

Signs and symptoms		HOURS					DAYS												
		1	2	3	5	24	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.
Sedation		+	+	+	+	+	+	+	+	+									
Dyspnoea		+	+	+	+	+	+	+	+	+	+								
Dacryorrhoea																			
Chromodacryorrhoea																			
Rinorrhoea																			
Epistaxis																			
Exophthalmos		+	+	+	+	+	+	+	+	+	+	+							
Salivation																			
Ruffled fur		+	+	+	+	+	+	+	+	+	+	+	+						
Pallor																			
Cyanosis																			
Diarrhoea																			
Body position (ventral)																			
Body position (lateral)																			
Body position (curved)		+	+	+	+	+	+	+	+	+	+								
Ataxia																			
Trismus																			
Tremor																			
Tonic clonic muscle spasms																			
Convulsions																			
SKIN IRRITATION	erythema						+ +	+ +	+ +	+ +	+ +	+ +	+	+					
	edema																		
	necrosis																		

+ = slight ++ = moderate

Exp. Toxicology Dossein
GU 2.1
TABLE 5 - SIGNS AND SYMPTOMS

Dose (mg/kg): 4000

Signs and symptoms		HOURS					DAYS												
		1	2	3	5	24	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.
Sedation		+	+	+	+	+	+	+	+	+									
Dyspnoea		+	+	+	+	+	+	+	+	+	+								
Dacryorrhoea																			
Chromodacryorrhoea																			
Rinorrhoea																			
Epistaxis																			
Exophthalmos		+	+	+	+	+	+	+	+	+	+	+							
Salivation																			
Ruffled fur		+	+	+	+	+	+	+	+	+	+	+	+						
Pallor																			
Cyanosis																			
Diarrhoea																			
Body position (ventral)																			
Body position (lateral)																			
Body position (curved)		+	+	+	+	+	+	+	+	+	+								
Ataxia																			
Trismus																			
Tremor																			
Tonic clonic muscle spasms																			
Convulsions																			
SKIN IRRITATION	erythema						++	++	++	++	++	++	++	+					
	edema																		
	necrosis																		

Key: + = slight, ++ = moderate, +++ = severe

4000 53

CD 5/106/8

I.D. # 07811

Industrial BIO-TEST Laboratories, Inc.
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

Ciba-Geigy Epoxy 0500

REPORT TO

CIBA-GEIGY CORPORATION

ACUTE DERMAL TOXICITY STUDY WITH EPOXY 0500 IN MALE ALBINO RABBITS

DECEMBER 17, 1975

IBT NO. 601-07231

Industrial BIO-TEST Laboratories, Inc.

REPORT TO

CIBA-GEIGY CORPORATION

Acute Dermal Toxicity Study - Male Albino Rabbits

Results

IBT No.: 601-07231
Test Material: Epoxy 0500
Physical Description: Brown liquid
Form Administered: Undiluted

Classification: Practically Nontoxic
Approximate Acute Dermal
Lethal Dose > 3,000 mg/kg

I. Mortality and Body Weights

Dose Level (mg/kg)	Animal Number	Individual Body Weights (kg) Test Day Number: 0	7	14	Number Dead / Number Tested	Percent Dead
300	1*	2.82	2.92	3.08	0/1	0
1,000	2*	2.70	2.72	3.08	0/1	0
3,000	3*	3.02	2.78	2.78	0/1	0

Note: The skin at the site of application was abraded.

II. Reactions and Pathology

No pharmacotoxic symptoms were noted in any of the rabbits following dermal exposure to Epoxy 0500.

The test material was moderately to severely irritating to the skin of the albino rabbit. Skin lesions at 24 hours were characterized by pale red to red, well-defined erythema and mild edema. Pale red to red, well-defined erythema mild edema and focal, second degree burns were noted at day 7, while superficial escharosis was observed at all test skin sites at day 14.

Necropsy examination revealed red lungs in all rabbits. No other gross pathologic alterations were noted except for the local skin changes as previously described.

Respectfully submitted,

INDUSTRIAL BIO-TEST LABORATORIES, INC.

Prepared by: Ken Lazzara
Ken Lazzara, B.S.
Technician
Acute Toxicity

Approved by: C. W. Mastri
C. W. Mastri, B.S.
Section Head, Acute Toxicity

chm

RANGE-FINDING ACUTE DERMAL TOXICITY STUDY - ALBINO RABBITS

Young adult albino rabbits of the New Zealand strain were used as test animals. All rabbits had been maintained under observation in the laboratory for at least 7 days prior to testing. During the pretest period, the animals were examined with respect to their general health and suitability as test animals. The rabbits were housed individually in suspended, wire-bottomed cages and maintained on a standard laboratory ration. Food and water were offered ad libitum.

Twenty-four hours prior to the dermal applications, the backs of the rabbits were shaved free of hair with electric clippers. The shaved area on each animal constituted about 30 percent of the total body surface area. The animals were then returned to their cages to await testing on the following day. The 24-hour waiting period allowed recovery of the stratum corneum from the disturbance which accompanied the close-clipping procedure and permitted healing of any microscopic abrasions possibly produced during the process.

A range of several dose levels was studied using 1 rabbit per level. The test site of each animal was covered by wrapping the trunk with impervious plastic sheeting which was securely taped in place. This plastic wrap insured close contact of the epidermis and test material. To prevent oral ingestion of the test material, each animal was fitted with a lightweight, flexible plastic collar which was worn throughout the observation period.

The test material remained in contact with the skin for 24 hours. At the end of this period, the plastic sheeting and all residual test material were removed. The test sites were examined for local skin reactions and the animals were returned to their cages. Observations for mortality, local skin reactions and behavioral abnormalities were continued for a total of 14 days following the skin applications. Initial, 7- and 14-day body weights were recorded. A necropsy examination was conducted on all animals.

At the end of the observation period, the approximate acute dermal lethal dose of the test material was determined. The test material was then assigned a classification. The classification system is presented below:

Approximate Acute Dermal Lethal Dose (Range of Values)	Classification	Probable Lethal Dose for a 70 kg Man in Commonly Used Measures
Less than 50 mg/kg	Extremely toxic	approximately 1/2 drops
50 - 500 mg/kg	Highly toxic	between 1/2 drops and 1 teaspoonful
500 - 5,000 mg/kg	Moderately toxic	between 1 teaspoonful and 1 pint
5,000 - 15,000 mg/kg	Slightly toxic	between 1 pint and 1 pint or 1 pound
15,000 - 50,000 mg/kg	Practically nontoxic	between 1 pint and 1 quart
Greater than 50,000 mg/kg	Relatively harmless	more than 1 quart

CO 5/103/75

ID# 13044

CIBA-GEIGY Limited
Basle, Switzerland

Exp. Toxicology Sisseln
GU 2.1

CAS 5026-74-4

REPORT 1M

SKIN IRRITATION IN THE RABBIT AFTER SINGLE APPLICATION OF

TK 12759

~~EPOXY 05-10~~ ARALDITE 0510

Project No.:	800658
Sponsor:	Plastics and Additives Division
Test material received:	March 28, 1980
Additional identification:	Physical appearance: yellow liquid Batch No : T-60011
The experiment started:	May 19, 1980
The experiment ended:	May 26, 1980
Study director:	L. Ullmann
Approved by:	Dr. med. vet. W. Gfeller
Reported:	June 2, 1980/wu
Archives:	Sisseln WST 452

p. 1/7

800658

1. Summary and Conclusion

Under the conditions of the present experiment TK 12759 was found to cause a marked irritation when applied to intact and abraded rabbit skin.

The calculated primary irritation index was 4.1.

2. Method

Concentration: conc.

The procedure used is described in the Proposed Guidelines of the United States Environmental Agency (EPA) § 163.81-5 "Primary dermal irritation study", Federal Register, Vol. 43, No. 163, August 22, 1978.

The test was performed on 6 male adult New Zealand White rabbits bred and raised on the premises weighing 2 to 3 kgs. They were housed individually in metal cages numbered by ear tags, were kept at a constant room temperature of 22 ± 2°C, at a relative humidity of 55 ± 10 % and on a 10 hours light cycle day. The animals received ad libitum standard rabbit food - NAFAG, No. 814, Gossau SG - and water. Prior to treatment they were adapted to our laboratories for a minimun of 4 days.

800658

Before treatment the entire back and the flank of the rabbits were shaved with an electric clipper and immediately before treatment the shaven skin on one side was slightly scarified with the help of a "Schröpfschnäpper", Aesculap, Switzerland. Gauze patches of 2.5 x2.5 cm laden with 0.5 ml of the test material were applied to the prepared abraded and intact skin.

The patches were covered with an impermeable material and were fastened to the body of the rabbit with adhesive tape. The dressings were removed after a 24 hour application.

The skin reaction was appraised upon removal and during an observation period of 7 days on the basis of the following evaluation scheme.

Score for skin irritation in rabbits

Erythema and eschar formation

No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Total possible erythema score	4

800658

Edema formation

No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4
Total possible edema score	4

3. Results

The results of the primary skin irritation test are reported in tables 1 and 2 and figure.

TK 12759 was found to cause a marked irritation when applied to intact and abraded rabbit skin.

800658

Calculation of the primary skin irritation index - Table 1

The scores read after 24 and 72 hours for erythema and edema for the intact as well as for the abraded skin were summed up and divided by 4. (FHSLA § 191.11)

Time after exposure hours	MEAN REACTION SCORE				
	erythema		edema		
	intact skin	abraded skin	intact skin	abraded skin	
24	2	2	1.8	2.5	
72	2	2	1.8	2.2	
total	4	+ 4	+ 3.6	+ 4.7	= 16.3

primary irritation index = 16.3 : 4 = 4.1

Assessment of irritation

0	= none
>0 - 1	= minimal
>1 - 2	= slight
>2 - 4	= moderate
>4 - 6	= marked
>6 - 8	= extreme

EVALUATION OF THE SKIN REACTIONS – TABLE 2

a = intact skin
b = abraded skin

After 24 hours

Sex		♂			♂		
Animal No.		350	351	352	353	354	355
Erythema	a	2	2	2	2	2	2
	b	2	2	2	2	2	2
Edema	a	2	2	2	2	2	1
	b	2	3	2	3	3	2
Total	a	4	4	4	4	4	3
	b	4	5	4	5	5	4
Mean	a	4			3.7		
	b	4.3			4.7		
Group mean	a	3.8					
	b	4.5					

After 48 hours

♂			♂		
350	351	352	353	354	355
2	2	2	2	2	2
2	2	2	2	2	2
2	2	2	2	2	1
3	2	2	2	2	2
4	4	4	4	4	3
5	4	4	4	4	4
4			3.7		
4.3			4		
3.8					
4.2					

After 72 hours

♂			♂		
350	351	352	353	354	355
2	2	2	2	2	2
2	2	2	2	2	2
2	2	2	2	2	1
3	2	2	2	2	2
4	4	4	4	4	3
5	4	4	4	4	4
4			3.7		
4.3			4		
3.8					
4.2					

After 4 days

Sex		♂			♂		
Animal No.		350	351	352	353	354	355
Erythema	a	2	2	2	2	2	2
	b	2	2	2	2	2	2
Edema	a	2	2	2	2	2	1
	b	2	2	2	2	2	1
Total	a	4	4	4	4	4	3
	b	4	4	4	4	4	3
Mean	a	4			3.7		
	b	4			3.7		
Group mean	a	3.8					
	b	3.8					

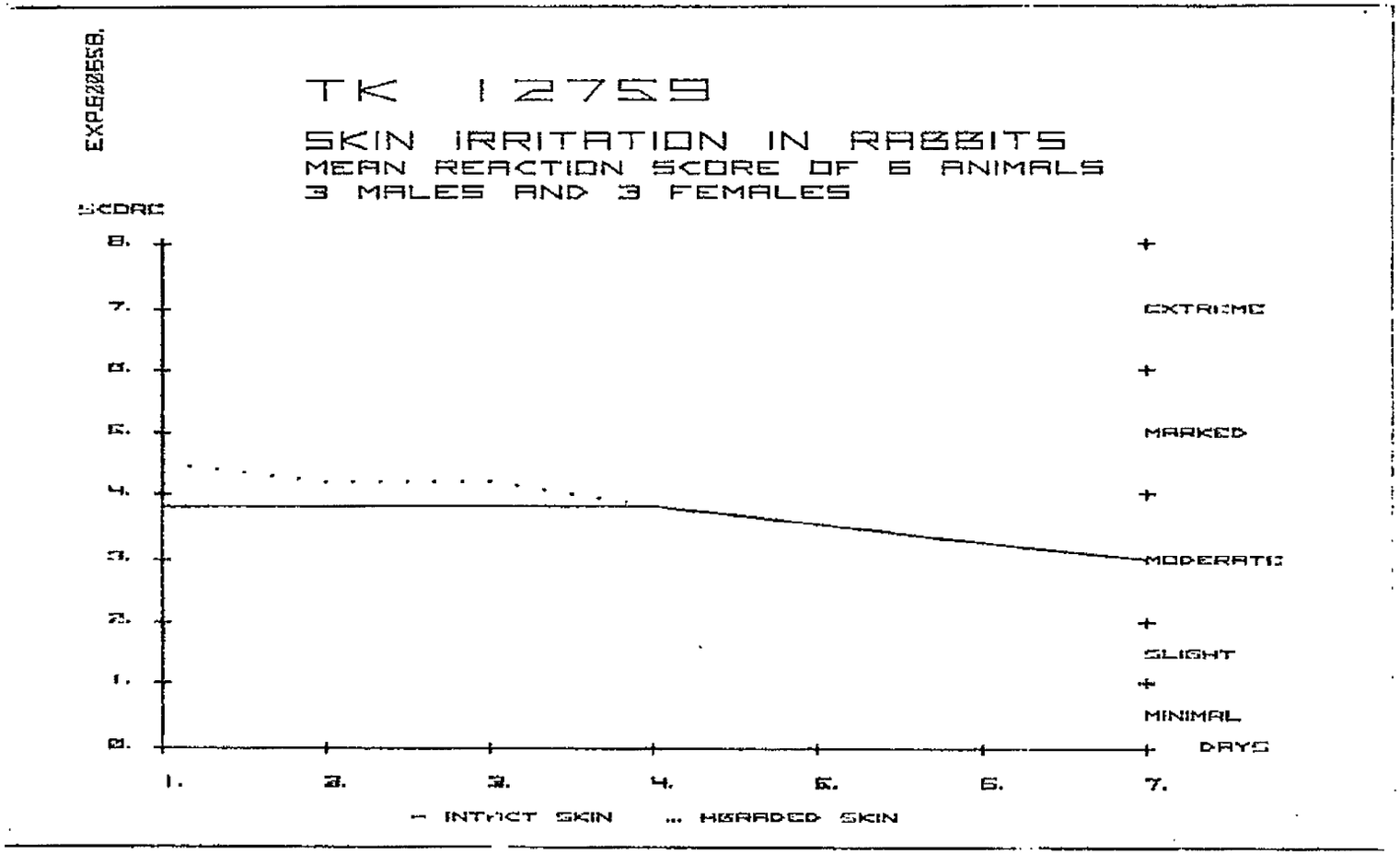
After 7 days

♂			♂		
350	351	352	353	354	355
2	2	2	2	2	2
2	2	2	2	2	2
1	1	1	1	1	1
1	1	1	1	1	1
3	3	3	3	3	3
3	3	3	3	3	3
3			3		
3			3		
3					
3					

After days

♂			♂		

400854


EXPERIMENTAL
TK 12759
SKIN IRRITATION IN RABBITS
MEAN REACTION SCORE OF 6 ANIMALS
3 MALES AND 3 FEMALES
SCORE
8.
7.
6.
5.
4.
3.
2.
1.
0.
EXTREME
MARKED
MODERATE
SLIGHT
MINIMAL
1.
2.
3.
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7.
DAYS
- INTACT SKIN ... ABRADED SKIN

CD 5/106/9

I.D. # 07812

Industrial BIO-TEST Laboratories, Inc.
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

Ciba-Geigy Epoxy 0500

REPORT TO

CIBA-GEIGY CORPORATION

EYE IRRITATION TEST WITH EPOXY 0500 IN ALBINO RABBITS

DECEMBER 17, 1975

IBT NO. 601-07231

REPORT TO

CIBA-GEIGY CORPORATION

TABLE

Eye Irritation Test - Albino Rabbits

Results

IBT No.. 601-07231
Test Material: Epoxy 0500
Physical Description: Brown liquid
Form Administered: Instillation (0.1 ml, undiluted)
Special Instructions: Unwashed eyes

Classification: Moderately Irritating
Maximum Mean
Irritation Score: 32.7/110.0

Tissue	Rabbit Number	1 Hour	24 Hours	48 Hours	72 Hours	7 Days	14 Days
Cornea (D-A)	1	0	10 (1-2)	10 (1-2)	0	0	0
Iris		5	5	5	0	0	0
Conjunctiva (R-S-D)		10 (1-1-3)	10 (2-1-2)	6 (1-1-1)	0	0	0
Total		15	25	21	0	0	0
Cornea (D-A)	2	0	15 (1-3)	15 (1-3)	10 (1-2)	5 (1-1),V	5 (1-1),V
Iris		0	5	5	5	0	0
Conjunctiva (R-S-D)		10 (1-1-3)	14 (3-2-3)	10 (2-2-1)	2 (1-0-0)	0	0
Total		10	34	30	17	5	5
Cornea (D-A)	3	0	20 (1-4)	20 (1-4)	20 (1-4)	40 (2-4),V	20 (1-4),V
Iris		5	5	5	5	5	5
Conjunctiva (R-S-D)		10 (1-1-3)	14 (2-2-3)	14 (2-2-3)	12 (2-2-2)	6 (1-1-1)	2 (1-0-0)
Total		15	39	39	37	51	27
Averages							
Cornea		0.0	15.0	15.0	10.0	15.0	8.3
Iris		3.3	5.0	5.0	3.3	1.7	1.5
Conjunctiva		10.0	12.7	10.0	4.7	2.0	0.7
Total		13.3	32.7	30.0	18.0	18.7	10.7

Cornea:
D = Density
A = Area
Corneal Score = D x A x 5
Maximum Score = 80

Iris:
Iris Score = Value x 5
Maximum Score = 10

Conjunctiva:
R = Redness
S = Swelling
D = Discharge
Conjunctival Score = (R+S+D) x 2

V = Vascularization

Respectfully submitted,

INDUSTRIAL BIO-TEST LABORATORIES, INC.

Report prepared by: Terry M. Davis
Terry M. Davis, B. S.
Assistant Toxicologist
Acute Toxicity

Approved by: C. W. Master
C. W. Master, B. S.
Section Head, Acute Toxicity

peb

CO: 5/103/93

ID# 11018

RESINS

TOXICITY

CIBA-GEIGY Limited
Basle, Switzerland

Exp. Toxicology Sissein
GU 2.1

ARALDITE 0510

REPORT ON

SKIN-SENSITIZING (CONTACT ALLERGENIC) EFFECT IN GUINEA PIGS OF

TK 12759

EPOXY 0510 (UK + USA)

Project No.:	800660
Sponsor:	Plastics and Additives Division
Test material received:	March 28, 1980
Additional identification:	Physical appearance: yellow liquid Batch No.: T-60011
The experiment started:	June 23, 1980
The experiment ended:	August 8, 1980
Study director:	L. Ullmann
Approved by:	Dr. med. vet. H. Gfeller
Reported:	August 26, 1980/Hu
Archives:	Sissein HST 452

PERMANENT RECORD FILE
TOXICOLOGY

800660

1. Summary and Conclusion

Under the experimental conditions employed, significant differences between the test group and the vehicle-treated controls were only seen after epidermal challenge application of TK 12759.

TK 12759 is therefore considered to possess skin-sensitizing (contact allergenic) potential in albino-guinea pigs.

2. Method

Concentration:	0.1 %
Vehicle:	propylene glycol

The optimization test[1] was used, an intracutaneous sensitization procedure similar to the method recommended in the 'Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics" (1959), the US Association of Food and Drug Officials (AFDO).

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1 Maurer Th., Thomann P., Weirich E.G., Hess R.

- The Optimization test in the guinea pig
  Agents & Actions 5 (2), 174-179, 1975
- Predictive evaluation in animals of the contact allergenic potential of medically important substances
  Contact Dermatitis 4, 321-333, 1978
  Contact Dermatitis 5, 1-10, 1979

000660

a) Animals

The test was performed on groups of 10 male and 10 female guinea pigs of the Pirbright White strain bred on the premises and weighing between 370 to 460 grams. The animals were housed individually in Macrolon cages, type 3, identified with individual ear tags, kept at a constant room temperature of 22 + 2°C, at a relative humidity of 55 +10 % and a 10 hours light cycle day. The animals received ad libitum standard guinea pig pellets - NAFAG, No. 830, Gossau SG, Switzerland - and water. Bodyweights were recorded immediately before starting the experiment (control values) and at termination of the study.

b) Testing procedure

During the induction period the animals received one injection every second day (except weekends) to a total of 10 intracutaneous injections of a freshly prepared 0.1 % dilution of TK 12759 in propylene glycol. One control group was treated with the vehicle alone ("negative control").

On the first day, injections of 0.1 ml were administered into the shaven skin of the right flank and on the back, while on the following days a single intracutaneous injection was given into the back.

During the second and third week of the induction period the test material was incorporated in a mixture of the normal vehicle with complete Bacto Adjuvant[2].

(vehicle : adjuvant = 1 : 1).

Fourteen days after the last sensitizing injection, a challenge injection of 0.1 ml of a freshly prepared 0.1 % dilution of TK 12759 in propylene glycol was administered into the skin of the left flank.

Twenty-four hours after each injection during the first week of the induction period and 24 hours after the challenge injection the reactions were recorded.

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2 Bacto Adjuvant, complete Freud (Difco, Michigan, USA).

800660

The two largest perpendicular diameters (in mm) and the increase in the skin- fold thickness (in mm) were measured and by multiplication of these values the "reaction volume" was obtained (in μl) for each reading from each animal. The mean volume plus one standard deviation of the induction reactions observed in the individual animal in the first week was taken as representing the skin irritation "threshold" for each animal. Any challenge reaction greater than this threshold value in the induction period was graded as an allergic reaction and the animal termed "positive". The number of 'positive" animals in the test group was compared with the number of animals in the control group (treated with the vehicle alone) that showed a non-specific reaction of at least the same magnitude ("negative control")[3].

Ten days after the intracutaneous challenge injection a subirritant dose of the test compound was applied epicutaneously under occlusive dressings which were left in place for 24 hours.The skin irritation was recorded according to Draize (described in the 'Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics" 1959 of the US Association of Food and Drug Officials (AFDO) 24 hours after removal of the dressings. For irritation score see table below

Score for skin irritation

Erythema and eschar formation

No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beed redness) to slight eschar formation (injuries in depth)	4
Total possible erythema score	4

Edema formation

No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges) of area well defined by definite raising	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4
Total possible edema score	4

3. Results

Table 1 summarizes the incidence of positive reactors after intradermal and epidermal challenge application, as well as the mean bodyweights of the animals and standard deviation.

Tables 2 and 3 show the reaction volumes for individual animals of the test and control group.

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3 The exact Fisher test for comparison of the basic probability of two binominal distributions; L. Sachs, Statistische Auswertungsmethoden, Thieme Verlag, Stuttgart, 1971. A probability of ≤ 0.01 was considered to indicate a significant difference.

800660

Table 1

A) Incidence of positive animals per group after intradermal challenge injection

	No. of positive animals / No. of treated animals	P
Vehicle alone	4/20	
TK 12759	8/20	0.15042

B) Incidence of positive animals per group after occlusive epicutaneous application

	No. of positive animals / No. of treated animals	P
Vehicle alone	0/20	
TK 12759 5 % in Vaseline	20/20	< 0.01

C) Challenge reactions after occlusive epicutaneous administration of the test material

Erythema score (Draize Score) 24 hours after removal of the dressing.

Animal No. m	2361	2362	2363	2364	2365	2366	2367	2368	2369	2370
	2	2	2	2	2	1	2	2	2	2

Animal No. f	2371	2372	2373	2374	2375	2376	2377	2378	2379	2380
	2	2	2	1	2	2	2	2	2	2

D) Mean Bodyweights and Standard Deviation (g)

	Vehicle control		TK 12759	
	male	female	male	female
pre test	429/41	407/42	434/22	414/29
end of test	579/44	525/35	614/47	536/23
Mean body weight gain	150	118	180	122

80 06 60

Table 2 : Reaction volumes (µl) after intradermal injection of propylene glycol

Animal No.	FIRST WEEK OF TESTING 1st ing	2nd ing	3rd ing	4th ing	mean ($\bar{x}$)	standard deviation (s)	($\bar{x}$ + s)	after skin sensitization	+ = positive reactor
2341 ♂	225	120	264	152	190	66	256	203	—
2342	200	130	293	96	155	93	248	132	—
2343	216	187	58	132	162	67	229	215	—
2344	110	99	140	90	110	22	132	112	+
2345	245	195	150	224	204	41	245	169	—
2346	230	245	169	96	155	68	253	252	—
2347	120	96	72	110	99	21	120	134	+
2348	137	137	114	150	162	25	187	156	—
2349	173	158	140	110	145	27	172	120	—
2350	130	130	273	192	181	68	249	73	—
2351 ♀	187	324	158	112	195	91	286	120	—
2352	115	88	144	110	114	23	137	132	—
2353	121	110	145	77	113	28	141	203	+
2354	187	80	202	77	136	67	203	120	—
2355	90	170	189	110	139	46	185	150	—
2356	168	110	135	100	128	30	158	172	+
2357	120	70	158	80	107	40	147	100	—
2358	153	45	168	77	112	60	172	145	—
2359	120	132	296	134	170	84	254	120	—
2360	96	168	169	80	103	39	152	66	—
Group-mean	152	139	173	147				147	4/20

818

80 06 60

Table 3 : Reaction volumes (ul) after intradermal injection of TK 12759

Animal No.	FIRST WEEK OF TESTING 1st ing	2nd ing	3rd ing	4th ing	mean ($\bar{x}$)	standard deviation (s)	($\bar{x}$ + s)	after skin sensitization	+ = positive reactor
2361 ♂	120	156	140	110	146	21	167	414	+
2362	90	90	276	70	131	97	228	192	—
2363	229	100	90	96	129	67	196	156	—
2364	187	346	240	90	201	93	294	273	—
2365	77	135	96	120	107	26	133	200	+
2366	230	245	640	185	325	211	536	145	—
2367	100	153	150	150	138	25	163	158	—
2368	170	122	180	130	150	29	179	306	+
2369	144	106	274	180	176	72	248	371	+
2370	140	110	172	220	160	47	207	312	+
2371 ♀	109	160	414	100	196	148	344	273	—
2372	237	0	172	300	177	129	306	243	—
2373	90	70	288	29	132	104	236	109	—
2374	162	170	215	90	159	52	211	200	—
2375	97	130	234	56	129	76	205	399	+
2376	130	127	0	90	87	61	148	185	+
2377	720	190	120	150	295	285	580	157	—
2378	140	70	216	110	134	62	196	144	—
2379	110	110	144	100	116	19	135	314	+
2380	110	0	518	90	179	231	410	132	—
Group-mean	170	128	227	129				234	8/20

CD 5/103/88

I.D. #13045. RESINS TOXICITY

CIBA-GEIGY Limited
Basle, Switzerland

CAS 5026-74-4

RECEIVED
P & A TOX
CENTER

ARALDITE 0510

SALMONELLA AND ESCHERICHIA/LIVER MICROSOME TEST

with

TK 12 759

(Test for mutagenic properties in bacteria)

EPOXY 0510

GU 2.3

January 22, 1982

Salmonella and Escherichia/ Liver Microsome Test

No. of experiment: 810939
Batch No.: T 60011

SUMMARY AND CONCLUSIONS

TK 12 759 was tested for mutagenic effects on histidine-auxotrophic strains of Salmonella typhimurium and on a tryptophan-auxotrophic strain of E. coli. The investigations were performed with the following concentrations of the trial substance without and with microsomal activation: 5, 10, 50, 100, 500, 1000 and 5000 µg/0.1 ml.

These tests permit the detection of point mutations in bacteria induced by chemical substances. Any mutagenic effects of the substances are demonstrable on comparison of the number of bacteria in the treated and control cultures that have undergone back-mutation to histidine- or tryptophan-prototrophism. To ensure that mutagenic effects of metabolites of the test substance formed in mammals would also be detected, experiments were performed in which the cultures were additionally treated with an activation mixture (rat liver microsomes and co-factors) (lit.1,2,3).

In the experiments carried out without and with microsomal activation on strains TA 100, TA 1535 and WP2 uvrA treatment with TK 12 759 led to an increase in the number of back-mutant colonies.

An increase in the number of back-mutant colonies was also observed in the experiment without activation on strain TA 98.

TK 12 759 and its metabolites, formed as a result of microsomal activation, thus exerted a mutagenic action in this test system.

Study director: (Dr. P. Arni)
Date: January 21, 1982

Report reviewed and approved by: (Prof.Dr.D.Müller)
(Head of Experimental Pathology)
Date: January 22, 1982

CIBA-GEIGY Limited
Basle, Switzerland
Protection of Health and Environment
Toxicology

PROCEDURE

The tests were carried out in accordance with the method proposed by AMES et al. (lit.1,2,3) and with the modifications described by the Ministry of Labour of Japan.

The bacteria on which the tests were performed were the histidine-auxotrophic TA 98, TA 100, TA 1535, TA 1537 and TA 1538, strains of Salmonella typhimurium and the tryptophan-auxotrophic strain E. coli WP2 uvrA.

The tests were performed with the following concentrations of the trial substance without and with microsomal activation: 5, 10, 50, 100, 500, 1000 and 5000 µg/0.1 ml. The substance was dissolved in DMSO (Merck, Darmstadt, Germany). DMSO alone was used for the negative controls (the substances and vehicles used for the positive controls are indicated below). Each Petri dish contained: 1) approx. 20 ml of minimum agar (Agar, Difco Laboratories, Detroit, Michigan, U.S.A., plus salts (Vogel-Bonner Medium E) and glucose), 2) 0.1 ml of the solution of the test substance or the vehicle and 0.1 ml of a bacterial culture (in nutrient broth, Difco Laboratories, Detroit, Michigan, U.S.A., 0.8% plus 0.5% NaCl) in 2.0 ml of soft agar. The soft agar was composed of: 100 ml of 0.6% agar solution with 0.6% NaCl and 10 ml of a solution of l-histidine, 0.5 mM (Fluka, Buchs, Switzerland) and +biotin 0.5 mM (Fluka, Buchs, Switzerland). In the experiments in which the substance was metabolically activated, 0.5 ml of an activation mixture was added also (lit.1,2,3). 1 ml activation mixture contained: 0.1 ml S9 fraction of liver from rats induced with Aroclor 1254 (Analabs., Inc., North Haven, Connecticut, U.S.A.) and 0.9 ml of a solution of co-factors.

Positive control experiments without microsomal activation were carried out simultaneously with the following substances: 1) for strains TA 98 and TA 1538: 2-nitrofluorene (Fluka, Buchs, Switzer-

land), 1 and 2 µg/0.1 ml DMSO; 2) for strains TA 100 and TA 1535: N-ethyl-N'-nitro-N-nitrosoguanidine (Sigma, Saint Louis, Missouri, U.S.A.), 3 and 5 µg/0.1 ml DMSO; 3) for strain TA 1537: 9(5)aminoacridine hydrochloride monohydrate (Fluka, Buchs, Switzerland), 40 and 80 µg/0.1 ml DMSO; 4) for strain E. coli WP2 uvrA: N-ethyl-N'-nitro-N-nitrosoguanidine (Sigma, Saint Louis, Missouri, U.S.A.), 2 and 5 µg/0.1 ml DMSO. The positive control experiments with microsomal activation were performed with 2-aminoanthracene (EGA-Chemie, Steinheim, Germany) with the following concentrations (in DMSO): 1) for strains TA 98, TA 100 and TA 1538: 0.5 and 1 µg/0.1 ml; 2) for strains TA 1535 and TA 1537: 2 and 4 µg/0.1 ml; 3) for strain E. coli WP2 uvrA: 40 and 80 µg/0.1 ml.

In the experiments without and with the addition of microsomal activation mixture three Petri dishes were prepared per strain and per group (i.e. per concentration or per control group).

The plates were incubated for about 48 hours at 37°C in darkness.

When the colonies had been counted, the arithmetic mean was calculated. The test substance is generally considered to be non-mutagenic if the colony count in relation to the negative control is not doubled at any concentration (lit.3).

RESULTS

(see Tables 1 to 5)

In the experiments performed without microsomal activation, treament with IK 12 759 led to an increase in the number of back-mutant colonies of strains TA 98, TA 100, TA 1535 and WP2 uvrA. This effect was observed at the concentrations of 50 or 100 µg/0.1 ml and above (strains TA 1535 and TA 100 respectively) and at the concentrations of 500 and 1000 µg/0.1 ml (strains TA 98 and WP2 uvrA).

In the experiments performed with microsomal activation this effect was observed at the concentrations of 50 or 100 µg/0.1 ml and above (strains TA 1535 and TA 100 respectively) and at the concentrations of 1000 and 5000 µg/0.1 ml (strain WP2 uvrA).

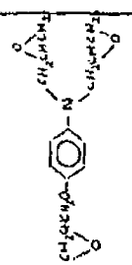
[1] AMES, B.N., F.D. LEE, and W.E. DURSTON (1973), An Improved Bacterial Test System for the Detection and Classification of Mutagens and Carcinogens.
Proc. Natl. Acad. Sci. USA 70, 782-786.

[2] AMES, B.N., W.E. DURSTON, E. YAMASAKI, and F.D. LEE (1973), Carcinogens are Mutagens: A Simple Test System Combining Liver Homogenates for Activation and Bacteria for Detection.
Proc. Natl. Acad. Sci. USA 70, 2281-2285.

[3] AMES, B.N., J. McCANN, and E. YAMASAKI (1975), Methods for Detecting Carcinogens and Mutagens with the Salmonella/Mammalian-Microsome Mutagenicity Test.
Mutation Res. 31, 347-364.

SUMMARIZED MUTAGENICITY TEST REPORT (MICROBIAL TEST)

1. GENERAL MATTERS

Name of new chemical substance (IUPAC naming)	Triglycidyl-p-aminophenol			
Common name or commercial name	EPOXY 0510	Physicochemical Properties	Molecular weight	≒ 300
Structural formula or Empirical formula (if not known, outline of manufacturing method)			State at room temperature	liquid
			Melting point	-- °C
			Boiling point	>200 °C
			Vapor pressure	unknown
			Soluble in	DMSO
Purity of test substance	wt%		Solubility	>5%
Name and content of impurity	wt%		Partition coefficiency	

2. BACTERIAL STRAINS

(1) Procurement

Obtained from	Date
Prof.B.Ames (Strains of S.typhimurium)	February, 1976
National collection of Industrial Bacteria, Aberdeen (Scotland) (Strain E.coli)	April, 1977

(2) Method of Preservation

Preservation Temperature	Composition	
-70°C to -80°C	Bacterial suspension	9.25 ml
	D M S O	0.75 ml
	Others ( )	---

3. S9 MIX

(1) Procurement of S9 (Encircle 1 or 2)

Division	Date Prepared
1. Made internally	October 26, 1981
2. Purchase (from)	

(2) Preservation Temperature of S9

Preservation Temperature	~70°C to ~80°C

(3) Preparation of S9 (Fill in where known when purchased)

Animals Used		Induction material	
Species, lineage	rat, Tif:RAIf(SPF)	Name	Aroclor 1254
Sex	male	Method of Administration	i.p. in sesame oil
Week age	approx. 7 weeks	Term and quantity of Administration (g/kg body weight)	single application 5 days prior sacrifice 0.5 g/kg
Weight	197 - 203 g		

(4) Composition of S9 Mix

Component:	in 1 ml of S9 Mix	Component:	in 1 ml of S9 Mix
S 9	0.1 ml	$NADP^+$	--- µmol
$Mg Cl_2$	8 µmol	NADPH	4 µmol
K Cl	33 µmol	Na-phosphate buffer	100 µmol
Glucose-6-phosphoric acid	5 µmol	Others: NADH	4 µmol
Glucose-6-phosphate dehydrated enzyme	---	distilled water	0.275 ml

4. PREPARATION OF TEST SOLUTION

Solvent	Method for suspension of refractory substances
DMSO	

5. GERM-FREE TEST (Encircle existing or absent)

Division	Bacterial growth (except object bacteria)	
Test solution	Existing	[Absent]
S9 Mix	Existing	[Absent]

6. TEST RESULTS

(1) Test Method

[1. Plate method]
2. Preincubation method

(2) Test Condition

		Plate Method	Preincubation Method
Composition	Bacterial suspension	0.1 ml	ml
	Test solution	0.1 ml	ml
	Na-phosphate buffer (for the test without activation)	0.5 ml	ml
	S9 Mix (for the test with metabolic activation)	0.5 ml	ml
	Soft gelatin solution	2.0 ml	ml
	Others	---	
Preincubation	Temperature		°C
	Time		minutes
Incubation	Temperature	37°C	°C
	Time	hours	hours

(3) Test Results: See attachment, Tables

7. OTHERS

Test Organization	Name	Experimental Pathology (Head: Prof.Dr.med.D.Müller) Protection of Health and Environment, Toxicology CIBA-GEIGY Limited, Basle, Switzerland			
	Address	CH-4002 Basle, Switzerland			
Tested by	Name	Christiane Braun			
	Experience	2½ years	School Career	Bachelor technical secondary school St.Louis, France	
and	Name	---			
	Experience	years	School Career		
Testing Work Supervisors: Technical assistant	Name	Eckhard Deparade			
	Experience	5½ years	School Career	Biological technical assistant Dr.Pfleger, Bamberg, FRG	
Study director	Name	Peter Arni			
	Experience	5½ years	School Career	Ph.D. in Biology University of Basle, Basle, Switzerland	
Test period	from November 17 to November 19, 1981				
Remarks	TK 12 759 displayed a mutagenic activity under the given test conditions.				

TABLE 1

RESULTS OF TESTS

Material	Concentration of testing sample (ug / plate)	Presence of S9 Mix	Back Mutation Number of colonies/plate (mean)					
			Base Conversion Type			Frame Shift Type		
			TA100	TA1535	WP2UVrA	TA98	TA1537	TA1538
Vehicle Control		-	158	12	17	19	4	11
TK 12 759	5	-	160	11	20	16	6	10
	10	-	179	13	17	20	5	10
	50	-	200	27	17	24	6	10
	100	-	479	39	25	20	4	9
	500	-	242	82	32	41	4	12
	1000	-	770	111	32	63	6	11
	5000	-	1393	55	11	25	2	1
Vehicle Control		+	149	8	14	37	6	22
TK 12 759	5	+	162	10	17	26	7	21
	10	+	162	12	17	32	5	21
	50	+	197	22	14	34	4	24
	100	+	235	43	18	41	5	30
	500	+	569	268	21	34	5	20
	1000	+	980	609	33	38	7	28
	5000	+	2041	1156	59	56	3	6

Positive Control								
Those which do not need S9 Mix	Name	N-ethyl-N'nitro-N-nitroso-guanidine			2-nitro-fluorene	9-amino-acridine	2-nitro-fluorene
	Concentration (ug / plate)	3	3	2	1	40	1
	No.of colonies/plate	1203	91*	261	227	56	254
	Concentration (ug / plate)	5	5	5	2	80	2
	No.of colonies/plate	894*	70*	923	467	426	504
Those which need S9 Mix	Name	2-aminoanthracene			2-aminoanthracene		
	Concentration (ug / plate)	0.5	2.0	40	0.5	2.0	0.5
	No.of colonies/plate	288	151	559	113	47	164
	Concentration (ug / plate)	1.0	4.0	80	1.0	4.0	1.0
	No.of colonies/plate	387	392	512	171	43	284

* A growth-inhibiting effect of the tested substance was observed.

TABLE 2

SALMONELLA AND ESCHERICHIA/LIVER MICROSOME MUTAGENICITY TEST
EXPERIMENTS WITHOUT MICROSOMAL ACTIVATION
NUMBER OF BACK-MUTANT COLONIES PER PLATE (ARITHMETIC MEAN)

No. of experiment: 810939 Test substance: TK 12 759

Date: November 19, 1981

STRAIN	TA 98	TA 100	TA 1535	TA 1537	TA 1538	WP2uvrA
Control	19	158	12	4	11	17
5 µg/0.1 ml	16	160	11	6	10	20
10 µg/0.1 ml	20	179	13	5	10	17
50 µg/0.1 ml	24	200	27	6	10	17
100 µg/0.1 ml	20	479	39	4	9	25
500 µg/0.1 ml	41	242	82	4	12	32
1000 µg/0.1 ml	63	770	111	6	11	32
5000 µg/0.1 ml	26	1393	55	2	1	11
Positive controls						
2-nitrofluorene						
Control	15					
1 µg/0.1 ml	227					
2 µg/0.1 ml	467					
N-ethyl-N´-nitro-N-nitrosoguanidine						
Control		176				
3 µg/0.1 ml		1203				
5 µg/0.1 ml		894				
N-ethyl-N´-nitro-N nitrosoguanidine						
Control			9			
3 µg/0.1 ml			91			
5 µg/0.1 ml			70			
9(5)aminoacridine-hydrochloride						
Control				5		
40 µg/0.1 ml				56		
80 µg/0.1 ml				426		
2-nitrofluorene						
Control					10	
1 µg/0.1 ml					254	
2 µg/0.1 ml					504	
N-ethyl-N´-nitro-N-nitrosoguanidine						
Control						15
2 µg/0.1 ml						261
5 µg/0.1 ml						923

TABLE 3

SALMONELLA AND ESCHERICHIA/LIVER MICROSOME MUTAGENICITY TEST
EXPERIMENTS WITH MICROSOMAL ACTIVATION
NUMBER OF BACK-MUTANT COLONIES PER PLATE (ARITHMETIC MEAN)

No. of experiment: 81U939 Test substance: TK 12 759

Date: November 19, 1981

STRAIN	TA 98	TA 100	TA 1535	TA 1537	TA 1538	WP2uvrA
Control	37	149	8	6	22	14
5 µg/0.1 ml	26	162	10	7	21	17
10 µg/0.1 ml	32	162	12	5	21	17
50 µg/0.1 ml	34	197	22	4	24	14
100 µg/0.1 ml	41	235	43	5	30	18
500 µg/0.1 ml	34	569	268	5	20	21
1000 µg/0.1 ml	38	980	609	7	28	33
5000 µg/0.1 ml	56	2041	1156	3	6	59
Positive controls						
2-aminoanthracene						
Control	40	138			23	
0.5 µg/0.1 ml	113	288			164	
1.0 µg/0.1 ml	171	387			284	
2-aminoanthracene						
Control			8	5		
2.0 µg/0.1 ml			151	47		
4.0 µg/0.1 ml			392	43		
2-aminoanthracene						
Control						15
40 µg/0.1 ml						559
80 µg/0.1 ml						512

TABLE 4

SALMONELLA AND ESCHERICHIA/LIVER MICROSOME MUTAGENICITY TEST
EXPERIMENTS WITHOUT MICROSOMAL ACTIVATION
NUMBER OF BACK-MUTANT COLONIES PER PLATE AND ARITHMETIC MEAN

No. of experiment: 810939 Test substance: TK 12 759

Date: November 19, 1981

STRAIN	TA 98	TA 100	TA 1535	TA 1537	TA 1538	WP2uvrA
Control	19	144	12	5	8	15
	20	173	9	2	12	12
	19	158	15	5	12	24
	19	158	12	4	11	17
5 µg/0.1 ml	15	138	14	6	9	25
	15	160	9	7	12	17
	17	183	9	4	9	19
	16	160	11	6	10	20
10 µg/0.1 ml	27	193	14	9	13	17
	16	161	16	3	5	17
	18	184	9	3	13	16
	20	179	13	5	10	17
50 µg/0.1 ml	33	204	31	5	13	27
	19	200	27	7	14	17
	20	196	24	5	2	6
	24	200	27	6	10	17
100 µg/0.1 ml	18	504	50	3	9	24
	20	464	28	8	12	28
	21	469	38	2	5	24
	20	479	39	4	9	25
500 µg/0.1 ml	49	256	104	5	16	20
	39	236	67	2	14	33
	36	234	76	5	5	44
	41	242	82	4	12	32
1000 µg/0.1 ml	65	723	121	7	16	28
	68	792	96	8	6	31
	55	796	115	4	12	37
	63	770	111	6	11	32
5000 µg/0.1 ml	5	1383	25	3	0	13
	32	1448	64	2	0	13
	42	1349	75	1	3	7
	26	1393	55	2	1	11
Positive controls						
Control	12	173	5	6	14	20
	15	171	5	3	5	12
	19	184	17	6	12	14
	15	176	9	5	10	15
Concentration A	249	1152	40	78	305	201
	240	1207	113	73	270	364
	193	1251	120	16	187	217
	227	1203	91	56	254	261
B	485	764	37	186	555	909
	512	951	88	392	470	1077
	405	968	85	699	488	782
	467	894	70	426	504	923

TABLE 5

SALMONELLA AND ESCHERICHIA/LIVER MICROSOME MUTAGENICITY TEST
EXPERIMENTS WITH MICROSOMAL ACTIVATION
NUMBER OF BACK-MUTANT COLONIES PER PLATE AND ARITHMETIC MEAN

No. of experiment: 810939 Test substance: TK 12 759

Date: November 19, 1981

STRAIN	TA 98	TA 100	TA 1535	TA 1537	TA 1538	WP2uvrA
Control	52	172	9	8	25	19
	25	147	3	5	21	8
	33	129	12	4	19	16
	37	149	8	6	22	14
5 µg/0.1 ml	26	176	18	7	18	16
	26	149	8	9	28	18
	26	162	5	5	17	16
	26	162	10	7	21	17
10 µg/0.1 ml	36	168	14	5	21	20
	25	159	8	6	26	19
	36	160	14	4	16	12
	32	162	12	5	21	17
50 µg/0.1 ml	44	208	28	5	19	16
	29	159	24	4	26	12
	30	224	15	3	26	15
	34	197	22	4	24	14
100 µg/0.1 ml	31	249	29	4	33	15
	37	207	61	3	21	20
	54	248	39	9	37	20
	41	235	43	5	30	18
500 µg/0.1 ml	28	603	230	5	18	15
	30	546	318	3	21	28
	43	558	257	7	21	21
	34	569	268	5	20	21
1000 µg/0.1 ml	29	1044	618	7	29	38
	45	972	688	8	43	28
	41	925	520	7	13	33
	38	980	609	7	28	33
5000 µg/0.1 ml	32	2163	1146	2	8	48
	73	2238	1145	2	7	76
	63	1723	1178	5	3	52
	56	2041	1156	3	6	59
Positive controls						
Control	48	163	6	3	25	13
	31	129	12	5	31	14
	40	123	7	7	13	17
	40	138	8	5	23	15
Concentration A	110	233	135	33	204	628
	115	341	174	45	172	524
	113	290	144	64	116	524
	113	288	151	47	164	559
B	193	393	349	51	309	428
	122	405	365	36	307	541
	199	364	461	43	236	568
	171	387	392	43	284	512

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I.D. # 16172

CIBA-GEIGY LIMITED
BASLE, SWITZERLAND

Test No.: 860921

Epoxy 0510

Protection of Health
and Environment

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Genetic Toxicology

R E P O R T

TRANSFORMATION/LIVER MICROSOME TEST

(In vitro test for transformation-inducing properties in mammalian fibroblasts with metabolic activation only)

Test material: TK 12 759 (EPOXY 0510)

Date: September 4, 1987

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Individual data	Tables	4-5
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G E N E R A L

Type of study:	Transformation/Liver-Microsome Test
Cell line:	House embryo fibroblasts BALB/3T3, clone A31-1-1, obtained from Dr.T.Kakunaga, National Cancer Inst., NIH, Bethesda, Maryland, U.S.A.
Purpose:	Detection of morphological changes due to transformations in mammalian cells in vitro induced by the metabolites of the test compound and/or by the test compound itself.
Test No.:	860921
Test material:	TK 12 759 (EPOXY 0510)
Batch No.:	T 60011
Purity:	Commercial grade
Validity (Expiration date):	Stable (ensured by sponsor)
Stability:	Ensured by sponsor
Treatment:	Test chemical in growth medium Solv.: DMSO (Dimethylsulfoxide, MERCK)
Concentrations:	0.11 - 900 ug/ml, range in toxicity test (14 conc.) 3.75, 7.5, 15, 30, 60 ug/ml in transformation test with metabolic activation
No. of cultures per group:	Fifteen

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Sponsor:	CIBA-GEIGY Limited, Plastics and Additives Division, Basle, Switzerland
Testing facility:	CIBA-GEIGY Limited, Basle, Switzerland, Laboratories of Genetic Toxicology, R-1066.
Personnel:	Study Director: Dr. A.Meyer
	Technical conduct: Miss D.Huberschwiller Mrs. L.Loos Miss M.Maurer
Starting date:	February 10, 1987
Date of study completion:	July 27, 1987
Reported and filed:	September 1987
Location of archives:	CIBA-GEIGY Limited, Basle, Switzerland, Archives of Genetic Toxicology.

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STUDY TITLE :

TK 12759 (EPOXY 0510)
TRANSFORMATION TEST WITH METABOLIC ACTIVATION ONLY

Sponsor : KA/AD QAI Name : BALTISBERGER D.
Test No. : 860931
Study director : MEYER A.DR.

THE QUALITY ASSURANCE INSPECTION(S) WAS (WERE) PERFORMED ON THE FOLLOWING DATE(S)

1 05.02.87 2 10.02.87
3 26.02.87 4 02.09.87 FINAL REPORT AUDIT

THE FINDINGS WERE REPORTED TO THE STUDY DIRECTOR AND TO THE MANAGEMENT ON THE FOLLOWING DATE(S):

1 05.02.87 2 13.02.87
3 27.02.87 4 02.09.87

QSE/QAU Manager : Dr. H. J. Weideli

.....
Signature

02.09.87
Date

Distribution : 0 Study director 0 GU 1.1 File

SUMMARY AND CONCLUSIONS

TK 12 759 (EPOXY 0510) was tested for transformation inducing effects on mouse fibroblasts (BALB/3T3) in vitro with metabolic activation. The investigations were performed with concentrations of 3.75, 7.5, 15, 30 and 60 ug/ml.

This test system permits the morphological detection of changes due to transformations in mammalian cells induced by chemical substances. BALB/3T3 cells multiply in culture until a monolayer is formed, after which no further division and growth occur. Treatment of the cells with chemical carcinogens gives rise to cellular transformations and subsequently to the development of visible colonies superimposed on the customary cell monolayer. There is ample evidence that the appearance of stratified colonies in cell cultures treated with carcinogens is closely correlated with malignant transformation (BERWALD and SACHS, 1965[1)], MONDAL and HEIDELBERGER, 1970[2)], DiPAOLO et al., 1972[3)], KAKUNAGA, 1973[4)], LANG et al., 1980[5)]). To ensure that any transformative effects of metabolites of the test substance formed in mammals are detected, the experiment is performed in which the cultures are treated with the compound in the presence of an activation mixture (rat-liver microsomes and co-factors).

In the solvent control colonies of transformed cells were observed in three of 15 dishes, giving a transformation-frequency value of 1.53. In the untreated control colonies of transformed cells were detected in two of 13 dishes, giving a transformation-frequency value of 0.78. At the various tested concentrations of TK 12 759 (EPOXY 0510) (3.75, 7.5, 15, 30, 60 ug/ml) six, three, five, six and four dishes respectively, with colonies of transformed cells were detected, giving transformation-frequency values (i.e. number of transformed cells/10^4 surviving cells) of 2.74, 1.01, 1.76, 3.93 and 16.22, respectively.

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Statistical comparison of the results from the solvent control and from the various concentrations showing colonies of transformed cells revealed no significant differences (confidence limit = 5%).

In concurrent "positive control" experiments with 2-acetylaminofluorene (50 and 100 ug/ml), colonies of transformed cells were detected in twelve of 15, respectively in eleven of 14 dishes and the transformation-frequency values were 11.92 and 29.22. Statistical comparison of the results of the positive controls with that found in the solvent control revealed significant differences at both concentrations.

It is concluded that under the given experimental conditions no effects were obtained that must be interpreted as suggestive of a transformative property of TK 12 759 (EPOXY 0510) and its metabolites.

Study director: (Dr. A. Meyer)
Date: September 4, 1987

Report reviewed and approved by: (Dr. E. Puri)
Senior Scientist,
Genetic Toxicology
Date: September 4, 1987

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PROCEDURE

A) Preparation of the microsomal activation mixture

Rat liver microsomal fraction S9 is prepared from Aroclor 1254 (Analabs Inc., North Haven, Connecticut, U.S.A.) induced livers of male Tif:RAIf(SPF) rats. The final concentration of components of the activation mixture in the test is 0.16 mM NADP, 0.20 mM glucose-6-phosphate, 0.32 mM $MgCl_2$, 1.32 mM KCl, 1 mM $NaH_2PO_4.H_2O$ and 10 ul/ml S9-fraction. The activation mixture is added to the medium at a concentration of 10% in both the cytotoxicity test and the mutagenicity test.

B) Cytotoxicity test

A toxicity test is firstly performed in vitro to determine the highest concentration to be used in the transformation assay. The concentration of the test substance best suited as the highest for the transformation assay is that causing a 80% reduction in colony-forming ability in comparison with the negative (solvent, DMSO) control.

The BALB/3T3 cells used in this preliminary test are taken from cultures in an exponential phase of growth. Two days after inoculation of the cells, the cultures are exposed to the test substance for 24 hours in the presence of activation mixture. In the experiment, 14 concentrations each of the test substance, increasing by a factor of 2 from 0.11 to 900 ug/ml are applied and one solvent control. The substance was dissolved in DMSO, sterilised by filtration and applied in Eagle's Minimum Essential Medium containing 10% foetal bovine serum (EMEM10). The Petri dishes are incubated in a 5%-CO_2 atmosphere. The treatment is terminated by washing the cultures, followed by reincubation in fresh growth medium (EMEM10) for further five days.

At the end of the incubation period, the surviving colonies are fixed and stained with Giemsa solution and counted with the aid of a Colony Counter (Dr.N.Gerber-Original, Zürich, Switzerland) with

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counting probe and register. The reduction in colony-forming ability as a result of treatment with the test substance in the various concentrations is calculated from the number of colonies formed after treatment in relation to the number formed in the negative solvent control. From the results obtained, the concentration required to produce about a 80% reduction in colony-forming ability is calculated. This concentration serves as the highest in the transformation assay, and is applied together with further four lower concentrations, diminishing by a factor of 0.5.

C) Transformation assay

The transformation assay is performed on BALB/3T3 cells treated with the selected concentrations. The procedure employed is based on that reported by KAKUNAGA (1973)[4].

Forty-eight hours before exposure to the test substance, a series of Petri dishes (50 x 15 mm) is seeded with 5×10^3 cells per dish and incubated. In the experiment, fifteen dishes are then treated for each of the following conditions: In the metabolically activated cultures, five preselected concentrations of the test substance; two positive controls (2-acetylaminofluorene (Fluka AG, Buchs SG, Switzerland) 50 and 100 ug/ml); a negative control containing the solvent, and an untreated negative control. The duration of treatment is 24 hours. After removal of the test substance, the cells are washed and incubated for 4 weeks in the growth medium, which is replenished twice weekly. The experiment is terminated by fixing the cell monolayers with methanol and staining with Giemsa. The stained colonies of transformed cells are examined under the microscope and counted with the naked eye. Only so-called type III foci, showing predominantly spindle shaped cells that are randomly oriented and exhibit criss-cross arrays at the periphery of the foci, are scored.

Parallel to the transformation assay, a cell-viability control is carried out. For this purpose, 300 cells per Petri dish are seeded and treated as described above. Three dishes each are used for the

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different concentrations of the test substance and for the negative and positive controls. The incubation time for the viability control was five days after treatment of the cultures. The values obtained from the viability control are used to normalize the results from the transformation test (number of transformed cells/cells plated), i.e. to preclude errors due to the assumption of 100% viability of the cells seeded in cultures for the transformation test. Thus, the calculated values correspond to the number of foci per 10'000 viable cells, giving the transformation frequency.

The significance of differences between the treated and control cultures is tested by calculation of the fiducial limits for p according to the binomial-distribution model[6]. The number of dishes without colonies is compared with the number showing one or more colonies.

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RESULTS
(Tables 1 to 3)

In a preliminary toxicity test (Table 1), 14 concentrations increasing by a factor of 2 from 0.11 to 900 ug/ml were tested to determine the concentration causing a reduction of about 80% in colony-forming ability, this being the concentration best suited to serve as the highest in the transformation test with metabolic activation. In the case of TK 12 759 (EPOXY 0510), this concentration was 60 ug/ml.

The transformation test was carried out with 60 ug/ml as the highest concentration and further four lower concentrations, decreasing by a factor of 0.5 (Tables 2 and 3). In the solvent control, altogether five colonies of transformed cells were observed in three of 15 dishes, giving a transformation-frequency value of 1.53. In the untreated control, altogether two colonies of transformed cells were detected in two of 13 dishes, giving a transformation-frequency value of 0.78. At the various tested concentrations of TK 12 759 (EPOXY 0510) (3.75, 7.5, 15, 30, 60 ug/ml), colonies of transformed cells were detected in six of 15 dishes, in three of 14 dishes, in five of 15 dishes, in six of 13 dishes and in four of 15 dishes, giving transformation-frequency values (i.e. number of transformed cells/10^4 surviving cells) of 2.74, 1.01, 1.76, 3.93 and 16.22, respectively.

Statistical comparison of the results from the solvent control and from the various concentrations showing colonies of transformed cells revealed no significant differences (confidence limit = 5%).

In concurrent "positive control" experiments with 2-acetylaminofluorene (50 and 100 ug/ml), development of foci was detected in twelve of 15, respectively in eleven of 14 dishes, giving transformation-frequency values of 11.92 and 29.22. Statistical comparison of these values (number of dishes with transformed colonies) with that found in the solvent control revealed significant differences at both concentrations.

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Table 1 Test No.: 860921

TRANSFORMATION/LIVER MICROSOME TEST
(with metabolic activation)

Test substance: TK 12 759 (EPOXY 0510)

Toxicity test Number of colonies
Concentrations (ug/ml)

Dish No.	1.	2.	3.	Total	Mean	%
Control	91	113	107	311	103.7	100.0
0.110	113	137	130	380	126.7	122.2
0.220	114	105	150	369	123.0	118.6
0.439	130	106	147	383	127.7	123.2
0.879	114	102	129	345	115.0	110.9
1.758	122	105	150	377	125.7	121.2
3.516	102	129	111	342	114.0	110.0
7.031	109	123	118	350	116.7	112.5
14.063	89	66	79	234	78.0	75.2
28.125	56	57	59	172	57.3	55.3
56.250	13	16	12	41	13.7	13.2
112.500	0	0	0	0	0.0	0.0
225.000	0	0	0	0	0.0	0.0
450.000	0	0	0	0	0.0	0.0
900.000	0	0	0	0	0.0	0.0

80 % reduction in colony-forming ability,
calculated result: 59.8073 ug/ml

Highest concentration chosen for transformation test: 60 ug/ml

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Table 2

Test No.: 860921

TRANSFORMATION/LIVER MICROSOME TEST

(with metabolic activation)

Number of cells plated/dish : a) Viability control : 300
b) Transformation assay : 5000

		Viability control	Transformation assay		
				Transformation frequency	
		(%)	Number of transf. foci/dish	per plated cell (x 10^4)	per surviving cell (x 10^4)
Solvent control		43.7	0.333	0.667	1.53
Untreated control		39.4	0.154	0.308	0.78
2-AAF	50.0 ug/ml	16.8	1.000	2.000	11.92
2-AAF	100.0 ug/ml	7.3	1.071	2.143	29.22
TK 12 759					
3.750 ug/ml		39.0	0.533	1.067	2.74
7.500 ug/ml		42.3	0.214	0.429	1.01
15.000 ug/ml		37.8	0.333	0.667	1.76
30.000 ug/ml		31.3	0.615	1.231	3.93
60.000 ug/ml		4.1	0.333	0.667	16.22

Table 3 Test No.: 860921

TRANSFORMATION/LIVER MICROSOME TEST
(with metabolic activation)

Test substance: TK 12 759 (EPOXY 0510)

Statistical Evaluation

N = number of Petri dishes per dose-group

X = number of Petri dishes per dose-group with at least one focus

			N	X	signif. difference	p_r	p_l
Solvent Control			15	3		0.440	
Untreated Control			13	2			0.028
2-AAF	50	ug/ml	15	12	*		0.560
2-AAF	100	ug/ml	14	11	*		0.534
TK 12 759	3.75	ug/ml	15	6			0.191
	7.5	ug/ml	14	3			0.061
	15	ug/ml	15	5			0.142
	30	ug/ml	13	6			0.224
	60	ug/ml	15	4			0.097

* = Dose-groups significantly different from the vehicle control (5% level).

X is considered to be binomially distributed with parameters N and p (unknown). Formulae for the calculation are to be found in "Wissenschaftliche Tabellen Geigy"[7].

The upper confidence limit of the response parameter p of the binomial distribution of the negative control (solvent control) is designated p_r. In the treated groups, p_l denotes the lower confidence limit for p.

If p_l (for a treated group) is greater than p_r for the negative control, then there are significantly more Petri dishes in this group with at least one focus (5% level).

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Table.4

TRANSFORMATION/LIVER.MICROSOME.TEST

(with metabolic activation)

Test substance: TK 12 759 Test No. : 860921
Batch No. : T 60011 Solvent : DMSO
Test date : 24.02.1987 Pos. Control: 2-AAF

Number of transformed foci per dish scored

Number of cells plated per dish: 5000

Dish No.	Sol. contr.	Neg. contr.	Pos. control 50.0	100.0	Test substance (ug/ml) 3.750	7.500	15.00	30.00	60.00
1.	2	1	2	2	2	1	1	2	2
2.	2	1	2	2	2	1	1	2	1
3.	1	0	2	2	1	1	1	1	1
4.	0	0	1	2	1	0	1	1	1
5.	0	0	1	1	1	0	1	1	0
6.	0	0	1	1	1	0	0	1	0
7.	0	0	1	1	0	0	0	0	0
8.	0	0	1	1	0	0	0	0	0
9.	0	0	1	1	0	0	0	0	0
10.	0	0	1	1	0	0	0	0	0
11.	0	0	1	1	0	0	0	0	0
12.	0	0	1	0	0	0	0	0	0
13.	0	0	0	0	0	0	0	0	0
14.	0	K	0	0	0	0	0	K	0
15.	0	K	0	K	0	K	0	K	0
Mean	0.333	0.154	1.000	1.071	0.533	0.214	0.333	0.615	0.333

K : dish was contaminated and not scored

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Table 5

TRANSFORMATION/LIVER.MICROSOME .TEST

(with metabolic activation)

Test substance: TK 12 759
Batch No. : T 60011
Test date : 24.02.1987

Test No. : 860921
Solvent : DMSO
Pos. Control: 2-AAF

Viability control

Number of cells plated per dish: 300

	Number of colonies								
	Solvent control	Negative control	Pos. cont. (ug/ml) 50.00	100.00	Test substance (ug/ml) 3.750	7.500	15.00	30.00	60.00
Dish 1	116	105	44	20	110	130	112	111	14
Dish 2	133	126	55	27	112	137	118	81	13
Dish 3	144	124	52	19	129	114	110	90	10
Total	393	355	151	66	351	381	340	282	37
Mean	131.0	118.3	50.3	22.0	117.0	127.0	113.3	94.0	12.3
Viability (%)	43.7	39.4	16.8	7.3	39.0	42.3	37.8	31.3	4.1

K: dish was contaminated and not scored

Table 6 TRANSFORMATION ASSAY ON BALB / 3T3 CELLS

HISTORICAL CONTROLS
(without metabolic activation)

(Studies done in 1986)

Study No.	Neg. contr. (Medium)		Neg. contr. (Vehicle)		Positive control (20-Methylcholanthrene) 1.5 ug/ml		3.0 ug/ml	
	X/N	f	X/N	f	X/N	f	X/N	f
840254	0/15	0.0	2/15	0.77	9/15	53.3	11/15	37.8
840254/1	0/15	0.0	2/15	0.71	7/15	6.0	12/15	12.1
840781	0/14	0.0	0/15	0.0	2/14	0.6	8/15	2.5
850017	1/15	0.24	0/9	0.0	10/10	8.6	11/14	59.0
841044	1/15	0.33	0/14	0.0	5/15	4.1	7/15	9.4
841044/1	0/15	0.0	0/15	0.0	7/15	3.5	12/15	7.1
841044/2	2/14	0.44	0/15	0.0	10/15	2.2	9/15	2.7
860116	1/15	0.20	4/15	1.0	15/15	14.0	14/15	8.5
860117	1/15	0.09	0/15	0.0	14/14	2.3	10/15	1.9

HISTORICAL CONTROLS
(with metabolic activation)

(Studies done in 1986)

Study No.	Neg. contr. (Medium)		Neg. contr. (Vehicle)		Positive control (2-Acetylaminofluorene) 50 ug/ml		100 ug/ml	
	X/N	f	X/N	f	X/N	f	X/N	f
840254	2/15	2.71	0/14	0.0	7/15	33.7	5/15	31.3
840371	1/15	0.36	0/15	0.0	2/15	1.6	6/15	5.3
840367	2/15	0.87	1/15	0.41	11/15	29.6	5/15	10.8
850072	7/14	4.82	3/15	1.04	15/15	68.0	10/15	680.0
850371	0/15	0.0	0/15	0.0	2/14	9.5	9/12	187.5
840781	0/14	0.0	0/14	0.0	7/15	7.0	1/15	1.2
840369	0/15	0.0	0/12	0.0	7/15	12.0	5/14	11.0
850017	1/15	0.79	0/15	0.0	6/15	10.4	4/15	40.0
850017/1	0/10	0.0	0/15	0.0	5/14	6.1	8/13	12.8
841044	1/15	0.46	0/15	0.0	5/15	6.1	7/15	11.2
860116	0/15	0.0	1/15	0.67	6/15	31.8	8/15	40.0
860117	0/15	0.0	1/14	0.3	4/15	4.3	8/15	5.1

N = No. of Petri dishes per dose-group
X = No. of Petri dishes per dose-group with at least one focus
f = Transformation-frequency value per surviving cell (x 10^{-4})

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Verteiler:

HH. A. v.Schulthess (2x)
Dr. A. Meyer (2x)
Prof. D. Müller (1x)

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CIBA-GEIGY Limited
Basle, Switzerland

ARALDITE 0510

BALB/3T3 CELL TRANSFORMATION ASSAY

TK 12 759

Epoxy Resin 0510

(In vitro test for transformation-inducing properties in mammalian fibroblasts)

PERMANENT RECORD FILE
TOXICOLOGY

GU 2.3

January 18, 1982

BALB/3T3 Cell Transformation Assay No. of experiment: 801549
Batch No.: T-60011

SUMMARY AND CONCLUSIONS

TK 12 759 was tested for transformation-inducing effects on mouse fibroblasts (BALB/3T3) in vitro. The investigations were performed with concentrations of 6.50×10^{-5}, 3.25×10^{-5}, 1.63×10^{-5}, 0.81×10^{-5} and 0.41×10^{-5} µl/ml.

This test system permits the morphological detection of changes due to transformations in mammalian cells induced by chemical substances. BALB/3T3 cells multiply in culture until a monolayer is formed, after which no further division and growth occur. Treatment of the cells with chemical carcinogens gives rise to cellular transformations and subsequently to the development of visible colonies superimposed on the customary cell monolayer. There is ample evidence that the appearance of stratified colonies in cell cultures treated with carcinogens is closely correlated with malignant transformation (BERWALD and SACHS, 1965[1], MONDAL and HEIDELBERGER, 1970[2], DiPAOLO et al., 1972[3], KAKUNAGA, 1973[4] LANG et al., 1980[5].

In both negative controls, i.e. the vehicle-treated control and the untreated control, no colonies of transformed cells were detected, and the transformation-frequency value (= number of transformed cells/10^4 surviving cells) was accordingly zero. Likewise at all five concentrations of TK 12 759 (6.50, 3.25, 1.63, 0.81 and 0.41×10^{-5} µl/ml) no colonies of transformed cells were observed.

In the positive controls treated with methylcholanthrene (1.5 and 3.0 µg/ml), the transformation-frequency values were 10.3 and 78.0, respectively. Statistical comparison of the results from the negative controls and the two positive controls revealed highly significant differences.

It is concluded that under the given experimental conditions no effects were obtained that must be interpreted as suggestive of a transformative property of TK 12 759.

F. Strasser

Study director: (F.F. Strasser)
Date: January 15, 1982

Report reviewed and approved by: (Prof.Dr.D.Müller)
(Head of Experimental Pathology)
Date: January 18, 1982

CIBA-GEIGY Limited
Basle, Switzerland
Protection of Health and Environment
Toxicology

PROCEDURE

A toxicity test was first performed in vitro to determine the highest concentration to be used in the transformation assay. The concentration best suited as the highest for the transformation assay is that causing a 25% reduction in colony-forming ability.

The BALB/3T3 cells used in this preliminary test were taken from cultures in an exponential phase of growth. The substance was dissolved in DMSO and applied in Eagle's Minimum Essential Medium containing 10% foetal bovine serum (EMEM10). In a first toxicity test 14 concentrations, increasing by a factor of 2 from 0.123×10^{-3} to 1.0 µl/ml were used. Since the lowest concentration used brought a higher reduction in colony-forming ability than required, a second toxicity test had to be performed. In this test again 14 concentrations were used from 0.025×10^{-5} to 195.4×10^{-5} µl/ml. The petri dishes were incubated for 72 h in a 5% CO_2 atmosphere. The final concentration of the solvent in the medium was 1.0%.

After the removal of the test substance, the cells were washed and incubated in the same growth medium (EMEM10) for a further four days at 37°C in a 5% CO_2 atmosphere.

At the end of the incubation period, the surviving colonies were fixed and stained with Giemsa solution and counted with the aid of a Colony Counter (Dr.N.GERBER-ORIGINAL, Zürich, Switzerland) with counting probe and register. The reduction in colony-forming ability as a result of treatment with the test substance in various concentrations was calculated from the number of colonies formed after treatment in relation to the number formed in the negative control. From the results obtained, the concentrations required to produce about a 25% reduction in colony-forming ability was calculated. This concentration served as highest concentration in the transformation assay, and was applied together with a further four lower concentrations, diminishing by a factor of 0.5.

The transformation assay was performed on BALB/3T3 cells treated with the selected concentrations. The procedure employed is based on that reported by KAKUNAGA (1973)[4].

Forty-eight hours before exposure to the test substance, a series of petri dishes (60 x 15 mm) was seeded with 5×10^3 cells per dish (density 10^3 cells/ml; 5 ml/dish) and incubated. Fifteen dishes were then treated for each of the following conditions: five preselected concentrations of the test substance; two positive controls (methylcholanthrene 1.5 µg/ml and 3.0 µg/ml); a negative control containing the vehicle, and an untreated negative control. The petri dishes were incubated for 72 h. After removal of the test substance, the cells were washed and incubated for four weeks in the growth medium, which was replenished on the tenth and the seventeenth day. The experiment was terminated by fixing the cell monolayers with methanol and staining with Giemsa. The stained colonies of transformed cells were examined under the microscope and counted with the naked eye.

Parallel to the transformation assay, a cell-viability control was run out. For this purpose, 200 cells per 5 ml petri dish were seeded and treated as described above. Three dishes each were used for the different concentrations of the test substance and for the negative and positive controls. The incubation time for the viability control was three days after treatment. The values obtained from the viability control are used to normalize the results from the transformation test (number of transformed cells/cells plated), i.e. to preclude errors due to the assumption of 100% viability of the cells seeded in cultures for the transformation test. Thus, the calculated values correspond to the number of foci per 10,000 viable cells, giving the transformation frequency.

Statistics

The significance of differences between the treated and control cultures was tested by calculation of the fiducial limits for p according to the binomial-distribution model[6]. The number of dishes without colonies was compared with the number showing one or more colonies.

RESULTS

(Tables 1 and 2)

In two preliminary toxicity tests, concentrations between 0.025×10^{-5} to 1.0 µl/ml were tested to determine a concentration causing a reduction of about 25% in colony-forming ability, this being the concentration best suited to serve as the highest concentration in the transformation test. In the case of TK 12 759, this concentration is 6.5×10^{-5} µl/ml.

The transformation test was carried out with 6.5×10^{-5} µl/ml as the highest concentration and a further four lower concentrations, decreasing by a factor of 0.5. All five concentrations of TK 12 759 employed as well as the vehicle and the untreated controls did not give rise to the development of foci.

In the positive controls treated with methylcholanthrene (1.5 and 3.0 µg/ml) development of foci was detected in 8 of 15 and 14 of 15 dishes respectively, giving transformation-frequency values of 10.3 and 78.0. Statistical comparison of these values with the controls revealed a highly significant difference.

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Table 1

Test No.: 801549

IN VITRO TRANSFORMATION OF BALB/3T3 CELLS

Number of cells plated/dish: a) Viability control: 200
b) Transformation assay: 0.5×10^4

	Viability control	Transformation assay		
		Mean number of transf. foci/dish	Number of transf.cells / 10^4 cells plated	Transformation frequency: Number of transf.cells / 10^4 surviving cells
Vehicle Control	37.5	0	0	0
Untreated Control	37.3	0	0	0
Methylcholanthrene 1.5 µg/ml	11.7	0.60	1.20	10.3
Methylcholanthrene 3.0 µg/ml	6.84	2.67	5.34	78.0
TK 12 759 0.41×10^{-5} µl/ml	34.0	0	0	0
0.81×10^{-5} µl/ml	37.2	0	0	0
1.63×10^{-5} µl/ml	36.3	0	0	0
3.25×10^{-5} µl/ml	34.8	0	0	0
6.50×10^{-5} µl/ml	31.5	0	0	0

Table 1 Test No.: 801349

IN VITRO TRANSFORMATION OF BALB/3T3 CELLS

Test substance: TK 12 759

Statistical Evaluation

N = number of petri dishes per dose-group

X = number of petri dishes per dose-group with at least one focus

	N	X	signif. difference	p_r	p_1
Vehicle Control	13	0		0.206	
Untreated Control	15	0			0
Methylcholanthrene 1.5 µg/ml	15	8	*		0.300
Methylcholanthrene 3.0 µg/ml	15	14	*		0.721
TK 12 759 0.41×10^{-5} µl/ml	14	0			0
0.81×10^{-5} µl/ml	14	0			0
1.63×10^{-5} µl/ml	14	0			0
3.25×10^{-5} µl/ml	14	0			0
6.50×10^{-5} µl/ml	15	0			0

* = Dose-groups significantly different from the vehicle and untreated controls (5% level).

X is considered to be binomially distributed with parameters N and p (unknown). Formulae for the calculation are to be found in "Wissenschaftliche Tabellen, Documenta Geigy"[1].

The upper confidence limit of the response parameter p of the binomial distribution of the negative control (vehicle control) is designated p_r. In the treated groups, p_1 denotes the lower confidence limit for p.

If p_1 (for a treated group) is greater than p_r for the negative control, then there are significantly more petri dishes in this group with at least one focus (5% level).

Verteiler:

HH. Prof.D.Müller (2x)
F.F. Strasser (1x)
Dr. A. von Schulthess (2x)

5/103/89

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Basle, Switzerland

ARALDITE 0510

POINT MUTATION ASSAY WITH MOUSE LYMPHOMA CELLS

with

TK 12 759

(In vitro test for mutagenic properties in mammalian cells)

CG Epoxy Resin 0510

GU 2.3
November 17, 1981

PERMANENT RECORD FILE
TOXICOLOGY

Point mutation assay with mouse lymphoma cells (in vitro test)

No. of experiment: 810994
Batch No.: T600 11

SUMMARY AND CONCLUSIONS

TK 12 759 was tested for mutagenic effects on mouse lymphoma cells (L5178Y) in vitro. The investigations were performed with concentrations of 0.21 and 0.51 µg/ml.

This test system permits the detection of forward point mutations in mammalian cells induced by chemical substances. The gene changes are detected by any of three marker genes that are contained in the L5178Y-line of mouse lymphoma cells[1]. Mutagenic effects manifest themselves in the occurrence of mutants, expressed by resistance to any of three antimetabolites (methotrexate, cytosine arabinoside and thymidine). Mutagenic effects of the substances are demonstrable on comparison of the number of colonies in the treated and control cultures.

In the 4 and 18 hour toxicity test each a concentration producing an 80% cell-kill, best suited for mutagenicity testing, was determined and subsequently used in the mutagenicity tests.

In the 4 h mutagenicity experiment, comparison of the number of forward mutant colonies of the treated and control cells after exposure to the antimetabolites revealed no differences attrib-

utable to treatment with the substance. In the 18 h mutagenicity experiment the selection medium, containing thymidine, displayed an increase in the number of forward mutants in comparison with the control. The mutant factor obtained was 2.86 which is above threshold value.

In this in vitro-test system, under the conditions described, TK 12 759 thus displayed a weak mutagenic activity.

Study director: (F.F. Strasser)
Date: November 17, 1981

Report reviewed and approved by: (Prof.Dr.D.Müller)
(Head of Experimental Pathology)
Date: November 17, 1981

CIBA-GEIGY Limited
Basle, Switzerland
Protection of Health and Environment
Toxicology

PROCEDURE

A toxicity test was first performed in vitro to determine the concentrations to be used in the mutagenicity assay. The concentration best suited for mutagenicity testing are those causing approximately 80% cell-kill.

The L5178Y cells used in this assay were taken from cultures in an exponential phase of growth. The substance was prepared in DMSO solution and applied in Fischer's medium containing 10% horse serum in various doses ranging from 0.0003 to 10000 µg/ml, and the incubation was continued for 4 or 18 h in a 5% CO_2 atmosphere in 5-ml Falcon flasks. The final concentration of the solvent or vehicle control (DMSO) in medium was 1%.

After the removal of the test substance, samples were taken for determination of viability. For each concentration, eight tubes each containing 100 cells in semisolid agar were prepared and incubated for 10 days at 37°C in a CO_2 atmosphere.

At the end of the incubation periods colony counts were carried out and the number of colonies in the control was set at 100%. As a rule, from the results obtained, the concentrations required to produce an 80% cell-kill are calculated.

For the 4 h incubation as well as for the 18 h incubation an 80% cell-kill was obtained. Thus, in the mutagenicity experiments the concentrations of 0.51 µg/ml and of 0.21 µg/ml respectively were employed.

The mutagenicity test was carried out by treating L5178Y cells with the selected concentrations at a cell density of 10^6/ml in Falcon flasks. The exposure time was 4 h and 18 h respectively. After removal of the substance, the cells were incubated for three days, the cell density being adjusted daily to 2×10^5

cells/ml. At the end of the expression period, the cells were set up in culture tubes at a density of $4x10^5$ cells/5 ml in a semi-solid agar containing antimetabolites. The antimetabolites added were methotrexate (LEDERLE), thymidine (FLUKA) and cytosine arabinoside (SERVA).

Parallel with these cultures a cell-viability control was carried out. For this purpose 100 cells per 5 ml were seeded in agar of the same quality but without antimetabolites. The incubation time of the mutagenicity test cultures was 14 days for the 18-h test and 15 days for the 4-h test, that for cell-viability control 11 days. The values obtained from the viability control are used to normalize the results received from the mutagenicity test, i.e. to preclude a 100%-viability of the cells seeded in cultures of the mutagenicity test.

The calculated mutant frequency corresponds to the number of colonies per 100,000 cells. The mutation factor is calculated by dividing the mutant frequency of the treated cells by the mutant frequency of the control cells. The test substance is considered to be non-mutagenic if the mutation factor is not greater than 2.5.

RESULTS
(Table 1)

The cells were incubated together with the substance in a concentration of 0.51 µg/ml for four hours and of 0.21 µg/ml for 18 hours. These concentrations produced the required 80% cell-kill.

In the 4 h-mutagenicity experiment performed with TK 12 759 there was no difference in the number of forward mutant colonies between the treated and the control cells after exposure to the antimetabolites. In the experiment carried out with an incubation time of 18 hours the number of mutant colonies was elevated in the thymidine selection medium. Here the mutant factor was 2.86.

[1] FISCHER, G.A., LEE, S.Y. and CALABRESI, P.: Detection of chemical mutagens using a host-mediated assay (L5178Y) mutagenesis system.
Mutation Research 26, 501-511 (1974).

Table 1

Test No.: 810994

POINT MUTATION ASSAY WITH MOUSE LYMPHOMA CELLS (IN VITRO TEST)

Test substance	System	Dosage	Antimetabolites					
			Cytosine-arabinoside		Methotrexate		Thymidine	
			MF	MFF	MF	MFF	MF	MFF
TK 12 759	in vitro, 4h	0.51 µg/ml	0.64		29.39		37.92	
		Control	1.08	0.59	17.72	1.66	23.56	1.61
	in vitro, 18h	0.21 µg/ml	0.6		8.21		14.88	
		Control	1.02	0.59	6.92	1.19	5.2	2.86

MF = Mutant frequency per 100,000 cells.
MFF = Factor (MF dosage group/MF control).

Verteiler:

HH. Prof.D.Müller (2x)
P.F. Strasser (1x)
Dr. A. von Schulthess (2x)

CD 5/103/86

ID# 11070

ANALYTE 0510

MUTAGENICITY EVALUATION OF

MA-66

IN THE AMES SALMONELLA/MICROSOME PLATE TEST

FINAL REPORT

SUBMITTED TO:

CIBA-GEIGY
ARDSLEY, NEW YORK 10502

PERMANENT RECORD FILE
TOXICOLOGY

SUBMITTED BY:

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20795

LBI PROJECT NO. 20838

JULY 1978

Litton BIONETICS

MA-66 = C-G Epoxy Resin 0510
Batch R3020

I. SPONSOR: CIBA-GEIGY

II. MATERIAL*

A. Identification: Ma-66

B. Date Received: April 14, 1978

C. Physical Description: Viscous yellow liquid

III. TYPE OF ASSAY: Ames Salmonella/Microsome Plate Test

IV. PROTOCOL NO.: DMT-100

V. RESULTS

The results of this assay are presented in Table 1.

VI. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound was examined for mutagenic activity in a series of in vitro microbial assays employing Salmonella and Saccharomyces indicator organisms. The compound was tested directly and in the presence of liver microsomal enzyme preparations from Aroclor-induced rats.

The compound was tested over a series of concentrations such that there was evidence of some chemically-induced physiological effects at the high dose level. The low dose in all cases was below a concentration that demonstrated any toxic effect. The dose range employed for the evaluation of this compound was from 0.01 µl to 10 µl per plate.

The compound was toxic to the strain TA-98 at 10 µl per plate.

The results of the tests conducted on the compound in the absence of a metabolic activation system were positive with the strains TA-1535, TA-100 and D4.

The results of the tests conducted on the compound in the presence of a rat liver activation system were positive with the strains TA-1535, TA-100 and D4.

*Information was supplied by the sponsor. If information was not indicated by the sponsor, N.I. was entered.

VI. INTERPRETATION OF RESULTS AND CONCLUSIONS (Continued)

The test compound, Ma-66, exhibited genetic activity with the strains TA-1535, TA-100 and D4 strains in activation and nonactivation assays conducted in this evaluation and is considered as mutagenic under these test conditions. These tests indicate that the test compound does not require metabolic activation to cause genetic activity and that the compound in itself is mutagenic.

Submitted by:

Study Director

D. R. Jagannath, Ph.D. — Date 7.10.78

Section Chief
Mammalian Genetics
Department of Genetics
and Cell Biology

Reviewed by:

David Brusick, Ph.D. — Date 7/13/78

Director
Department of Genetics
and Cell Biology

V. RESULTS

TABLE 1

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: MA-66
B. SOLVENT: DMSO
C. TEST INITIATION DATE: JUN. 8, 1978
NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS (UL) OR MICROGRAMS (UG) PER PLATE.

TEST	SPECIES	TISSUE	REVERTANTS PER PLATE											
			TA-1535		TA-1537		TA-1538		TA-98		TA-100		D4*	
			1	2	1	2	1	2	1	2	1	2	1	2
NONACTIVATION														
SOLVENT CONTROL	---	---	12		14		8		29		89		28	
POSITIVE CONTROL**	---	---	2013		408		1764		1295		1249		409	
TEST COMPOUND														
0.010000 UL	---	---	19		18		6		20		124		34	
0.100000 UL	---	---	38		16		7		20		199		43	
1.000000 UL	---	---	147		14		5		36		813		64	
5.000000 UL	---	---	56		3		6		25		494		126	
10.000000 UL	---	---	12		2		5		0		459		140	
ACTIVATION														
SOLVENT CONTROL	RAT	LIVER	21		23		19		40		120		33	
POSITIVE CONTROL***	RAT	LIVER	429		456		1139		1357		784		31*	
TEST COMPOUND														
0.010000 UL	RAT	LIVER	51		24		16		38		135		41	
0.100000 UL	RAT	LIVER	181		24		10		42		313		42	
1.000000 UL	RAT	LIVER	674		15		16		48		814		43	
5.000000 UL	RAT	LIVER	457		9		10		32		982		127	
10.000000 UL	RAT	LIVER	140		10		2		45		327		111	

* TRY+ CONVERTANTS PER PLATE

** TA-1535 ETHYLMETHANE SULFONATE 10 UL/PLATE
TA-1537 QUINACRINE MUSTARD 10 UG/PLATE
TA-1538 2-NITROFLUORINE 10 UG/PLATE
TA-98 2-NITROFLUORINE 10 UG/PLATE
TA-100 ETHYLMETHANE SULFONATE 10 UL/PLATE
D4 ETHYLMETHANE SULFONATE 10 UL/PLATE
SOLVENT DMSO 50 UL/PLATE

*** TA-1535 2-ANTHRAMINE 2.5 UG/PLATE
TA-1537 2-ANTHRAMINE 2.5 UG/PLATE
TA-1538 2-ANTHRAMINE 2.5 UG/PLATE
TA-98 2-ANTHRAMINE 2.5 UG/PLATE
TA-100 2-ANTHRAMINE 2.5 UG/PLATE
D4 2-ANTHRAMINE 2.5 UG/PLATE
SOLVENT DMSO 50 UL/PLATE

* THERE IS NO KNOWN POSITIVE CONTROL COMPOUND THAT WORKS WITH THIS STRAIN IN THE ACTIVATION PLATE ASSAYS.

PROTOCOL

1. PURPOSE

The purpose of this study was to evaluate the test material for genetic activity in a microbial assay with and without the addition of mammalian metabolic activation preparations.

2. MATERIALS

A. Indicator Microorganisms

A description of strain verification is given in Standard Operating Procedure on page 10.

Salmonella typhimurium	TA-1535
	TA-1537
	TA-1538
	TA-98
	TA-100
Saccharomyces cerevisiae	D4

B. Activation System

1. Reaction mixture

Component	Final Concentration/ml
TPN (sodium salt)	4 µmol
Glucose-6-phosphate	5 µmol
Sodium phosphate (dibasic)	100 µmol
$MgCl_2$	8 µmol
KCl	33 µmol
Homogenate S9 fraction	0.1 ± .05 ml

2. S9 homogenate

A 9,000 x g supernatant was prepared from Sprague-Dawley adult male rat liver induced by Aroclor 1254 five days prior to kill according to the procedure of Ames et al. (1975). S9 samples were coded by lot number and assayed for milligrams protein per milliliter and relative P448/P450 activity by methods described in EBI Technical Data on Rat Liver S9 Product.

2. MATERIALS (Continued)

C. Positive Control Chemicals

The chemicals used for positive controls in the nonactivation and activation assays are given in Table 1 of Section V. Results.

D. Solvent

Either deionized water or dimethylsulfoxide (DMSO) was used to prepare stock solutions of solid materials. All dilutions of test materials were made in either deionized water or DMSO. The solvent employed and its concentration are recorded in Table 1 of Section V. Results.

3. EXPERIMENTAL DESIGN

A. Plate Test (Agar Incorporation)*

Approximately 10^8 cells from an overnight culture of each indicator strain were added to separate test tubes containing 2.0 ml molten agar supplemented with biotin and a trace of histidine. For nonactivation tests, at least 4 dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests, at least 4 dose levels of the test chemical were added to the appropriate tubes with cells. Just prior to pouring, an aliquot of reaction mixture (0.5 ml containing the 9,000 x g liver homogenate) was added to each of the activation overlay tubes, which were then mixed, and the contents poured over the surface of a minimal agar plate and allowed to solidify. The plates were incubated for 48 hr at 37°C and scored for the number of colonies growing on each plate. D4 yeast plates were incubated at 30°C (nonactivation) and 37°C (activation) for 3-5 days and then scored. The concentrations of all chemicals are given in Table 1 of Section V. Results. Positive and solvent controls using both directly active positive chemicals and those that require metabolic activation were run with each assay.

---

* Certain classes of chemicals known to be mutagens and carcinogens do not produce detectable responses using the standard Ames agar incorporation method. Some dialkyl nitrosamines and certain substituted hydrazines are mutagenic in suspension assays, but not in the plate assay. Chemicals of these classes should be screened in a suspension assay.

3. EXPERIMENTAL DESIGN (Continued)

B. Recording and Presenting Data

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were analyzed in a computer program and reported on a printout. The results are presented as revertants (or convertants for Q4) per plate for each indicator strain employed in the assay. The positive and solvent controls are provided as reference points. Other relevant data are provided on the computer printout.

4. EVALUATION CRITERIA

Plate test data consist of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test chemical and the cells are incubated in the overlay for 2 days, and a few cell divisions occur during the incubation period, the test is semiquantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test:

- The small number of cell divisions permits potential mutagens to act on replicating DNA, which is often more sensitive than nonreplicating DNA.
- The combined incubation of the compound and the cells in the overlay permits constant exposure of the indicator cells for 2 days.

A. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test chemical, the surviving population on the treatment plates is essentially the same as that on the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol normally employs several doses ranging over 2 or 3 log concentrations, the highest of these doses being selected to show slight toxicity as determined by subjective criteria.

B. Dose-Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. A factor that might modify dose-response results for a mutagen would be the

4. EVALUATION CRITERIA (Continued)

B. Dose-Response Phenomena

selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test chemical may kill any mutants that are induced, and the compound will not appear to be mutagenic.

C. Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct-acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assays. Negative controls consist of the test compound solvent in the overlay agar together with the other essential components. The negative control plate for each strain gives a reference point to which the test data are compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.

D. Evaluation Criteria for Ames Assay

Because the procedures used to evaluate the mutagenicity of the test chemical are semiquantitative, the criteria used to determine positive effects are inherently subjective and are based primarily on a historical data base. Most data sets are evaluated using the following criteria:

1. Strains TA-1535, TA-1537, and TA-1538

   If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the lowest increase equal to twice the solvent control value is considered to be mutagenic.

2. Strains TA-98, TA-100, and D4

   If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value for TA-100 and 2-3 times the solvent control value for strains TA-98 and D4 is considered to be mutagenic. For these strains, the dose-response increase should start at approximately the solvent control value.

4. EVALUATION CRITERIA (Continued)

D. Evaluation Criteria for Ames Assay

3. Pattern

Because TA-1535 and TA-100 are both derived from the same parental strain (G-46) and because TA-1538 and TA-98 are both derived from the same parental strain (D3052), there is a built-in redundancy in the microbial assay. In general the two strains of a set respond to the same mutagen and such a pattern is sought. It is also anticipated that if a given strain, e.g., TA-1537, responds to a mutagen in nonactivation tests, it will generally do so in activation tests (The converse of this relationship is not expected.). While similar response patterns are not required for all mutagens, they can be used to enhance the reliability of an evaluation decision.

4. Reproducibility

If a chemical produces a response in a single test that cannot be reproduced in one or more additional runs, the initial positive test data lose significance.

The preceding criteria are not absolute, and other extenuating factors may enter into a final evaluation decision. However, these criteria are applied to the majority of situations and are presented to aid those individuals not familiar with this procedure. As the data base is increased, the criteria for evaluation can be more firmly established.

E. Relationship between Mutagenicity and Carcinogenicity

It must be emphasized that the Ames Salmonella/Microsome Plate Test is not a definitive test for chemical carcinogens. It is recognized, however, that correlative and functional relationships have been demonstrated between these two endpoints. The results of comparative tests on 300 chemicals by McCann et al. (1975) show an extremely good correlation between results of microbial mutagenesis tests and in vivo rodent carcinogenesis assays.

All evaluations and interpretation of the data presented in this report are based only on the demonstration, or lack, of mutagenic activity.

REFERENCES

Ames, B.N., McCann, J. and Yamasaki, E. (1975). Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutation Res. 31, 347-364.

McCann, J., Choi, E., Yamasaki, E. and Ames, B.N. (1975). Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. Proc. Nat. Acad. Sci. 72, 5135-5139.

5. STANDARD OPERATING PROCEDURE

All data will be entered in ink (no pencil).

All changes or corrections in entries will be made with a single line through the change, and an explanation for the change must be written.

All calculations (weights, dilutions, dose calculations, etc.) will be shown on data records.

All data entries will be dated and initialed.

All laboratory operations will be written out in standard protocol manuals. These manuals will be present in each laboratory area.

Deviations from any established protocol will be described and justified.

Data will be stored in bound form (notebooks or binders). These bound data books will be reviewed by the appropriate Section Heads.

Chemicals submitted for testing will have date of receipt and initials of entering person.

Lot numbers for all reference mutagens, solvent, or other materials used in assays will be recorded.

Animal orders, receipts, and identification will be recorded and maintained such that each animal can be traced to the supplier and shipment. All animals on study will be properly identified.

A copy of the final report plus all raw data and support documents will be permanently stored in the archival system of Litton Bionetics, Inc.

Current curricula vitae and job descriptions will be maintained on all personnel involved in the study.

Salmonella strains will be routinely checked for the his, uvrB, rfa, and pKM 101 phenotypes. Only appropriately screened stock cultures will be used in chemical evaluations.

1535 +1M
1538 -98
1537 [illegible]

NO RAW DATA SUPPLIED AS REQUESTED IN LETTER OF 6/30/78

MA 67 - TA 1538 non-activated reported as negative. - Why?
or why not repeated as with MA 61

MA 66 & 67 - all solvent & positive controls are same except

activation assay

	TA 98	TA 100	D4
MA 66	40 / 1357	120 / 784	33 / 31+
MA 67	40 / 1307	119 / 784	39 / 31+

+ means no known positive control compound works with this strain in this assay

Why use it then?

inactivation assay

	TA 1538
MA 66	1764
MA 67	1764

~~MA 60~~

~~page 1 states conc. employed for evaluation was from 0.1 ug to 500 ug/plate~~
~~activation assay also run at 1000 ug~~

~~MA 60~~	D4 positive Control			Date Initiated
MA 60	ANTH 2.5 ug/plate	190	?	1/12
MA 61	DMNA 100 um/plate	190		1/12
MA 62	DMNA 100 um/plate	190	?	1/12
MA 63	DMNA 100 um/plate	190	?	1/12
	~~Corrected MA 62 report~~			
MA 64	ANTH 2.5 ug/plate	190		1/12
MA 65	DMNA 100 um/plate	190		1/12

~~Raw data comments~~

Corrected MA 62 report. raw data does not show that cpd was tested at 20 mg for 1535
TA 100 N.A. shows no conc. on raw data sheet for highest level used & [illegible] 1,5,1

AMES RAW DATA

TM# 306-MA50
" 306-MA53
TM# 309-MA58
" 309-MA59
MA60 - TM# 311
MA66 - TM# 315
MA67 - " " 315

MA-50 = (ARALDITE) XB 2793
Regular
Batch #291078 TM# 306

MA-53 = (ARALDITE) LSU 609 TM# 309
Diglycidyl Ethylamyl Hydantoin
Regular
Batch #SE 73-22 VPS 116 EP 1&2

MA-58 = (ARALDITE) LMB 894 TM# 309
Distilled

Lot #I25P147D

MA-59 = PC-206 TM# 309

Lot # 93

MA-60 = (ARALDITE) PO 9103
Distilled TM# 311

Batch #I 26T9C

MA-66 = C-G Epoxy Resin 0510

Batch #R3020 TM# 315

MA-67 = Indigo Powder TM# 315

DCT #80111

PERMANENT RECORD FILE
TOXICOLOGY

Litton BIONETICS

RUSH

CIBA-GEIGY Ma-50

11 21 77 JC 2542

viscous colorless liquid DMSO

IRL

CONCENTRATION µl/PLATE	BV RB-12/1	BV RB-12/1	MW RB-12/1	[illegible]	BV RB-12/1	[illegible]
SOLVENT CONTROL	21 ~~17~~ 18	~~17~~ 6	9 ~~4~~	28 ~~24~~ 40	~~109~~ 122 ~~108~~ 201	~~79~~ 81 82
POSITIVE CONTROL	943 —	508	962	1187 —	871 —	1125 —
0.001	22 —	5	14	25 —	138 —	88 —
0.01	23 —	2	9	22	114 —	92 —
0.1	24 —	3	14	28 —	100 —	78 —
1.0	35 —	2	11	20 —	177 —	78 —
5.0	15 16	2	1	27 42	104 163	118 79
10.0	— 2	—	—	— 36	— 130	— 63
SOLVENT CONTROL	17 ~~14~~ 22	~~17~~ 6	~~12~~	40 ~~43~~ 40	~~[illegible]~~ 307 267	~~[illegible]~~ 85 97
POSITIVE CONTROL	124 —	175	815	1042 —	1873 —	187 —
0.001	21 —	5	20	22 —	235 —	87 —
0.01	17 —	7	17	24 —	280 —	75 —
0.1	23 —	7	16	44 36	263 247	63 —
1.0	40 26	3	12	16 45	343 [illegible]	85 —
5.0	11 25	6	6	20 37	317 271	101 84
10.0	— 24	—	—	— 3	— 82	— 72

[illegible] 11/2 [illegible] 24 [illegible] 77 by: [illegible]

[illegible] BV RB 12/1 BV RB 12/1 MW RB-12/1 [illegible] BV RB 12/1 [illegible]

[illegible]

Negative [illegible] 12/13

RUSH

Ma-53

2545

IRL

SOLVENT CONTROL						
POSITIVE CONTROL						
0.001						
0.01						
0.1						
1.0						
5.0						
SOLVENT CONTROL						
0.001						
0.01						
0.1						
1.0						
5.0						
10.0						
20.0						

Positive is

LBI COMMERCIAL PLATE ASSAY REPORT FORM

CLIENT Ciba-Hugy CHEMICAL NAME OR CODE NO. Ma-53

DATE RECEIVED mo.: 12 day: 21 yr.: 77 by: [illegible] ASSAY NO. 2689

TEST CONDITIONS

PHYSICAL DESCRIPTION viscous colorless liquid SOLVENT DMSO

ACTIVATION SYSTEM EMPLOYED ICL

OTHER CONSIDERATIONS

TEST RESULTS

CONCENTRATION	INDICATOR ORGANISMS (REVERTANTS/PLATE)											
µl /PLATE	TA-1535		TA-1537		TA-1538		TA-98		TA-100		D4*	
S9 MIX	15	17	~~18~~		~~16~~		41		~~95~~	133	52	
SOLVENT CONTROL	~~14~~	~~16~~	16		28		~~43~~		104	~~64~~	~~43~~	
POSITIVE CONTROL	624		528		1450		1435		822		622	
0.001	12		11		19		44		112		34	
0.01	12		9		21		41		114		47	
0.1	13		9		20		34		112		53	
1.0	112	16	12		16		40		112	164	41	
5.0	109	168	12		21		35		148	412	59	
10.0		221								739		
+ S9 MIX	~~14~~		20		22		34		161	111	~~122~~	
SOLVENT CONTROL	24	19	~~24~~		~~22~~		~~48~~		~~159~~	~~162~~	147	
POSITIVE CONTROL	422		312		1173		1240		227		180	
0.001	14		15		16		39		106		139	
0.01	19		20		20		29		105		164	
0.1	29		13		14		35		128		154	
1.0	37	13	27		16		36		129	197	175	
5.0	238	181	25		11		38		354	522	140	
10.0	230	143								733		

*Try+ convertants per plate TEST DATE mo.: 1 day: 10 yr.: 78 by: [illegible]

EVALUATION/CONCLUSION SUBMITTED BY:

Possible do
85 and 100 in
ICL / NA
DW 3/21

Solvent controls closest to the test values and within the normal spontaneous range were selected. The other value was crossed out.

LBI COMMERCIAL PLATE ASSAY REPORT FORM

CLIENT Ciba-Geigy CHEMICAL NAME OR CODE NO. Ma-59

DATE RECEIVED mo.: 12 day: 21 yr.: 77 by: [illegible] ASSAY NO. 2690

TEST CONDITIONS

PHYSICAL DESCRIPTION light amber liquid SOLVENT DMSO

ACTIVATION SYSTEM EMPLOYED IRL

OTHER CONSIDERATIONS

TEST RESULTS

CONCENTRATION	INDICATOR ORGANISMS (REVERTANTS/PLATE)					
µl /PLATE	TA-1535	TA-1537	TA-1538	TA-98	TA-100	WB 1/16 D4*
-S9 MIX	15	~~15~~	~~16~~	~~44~~	~~95~~	52
SOLVENT CONTROL	~~14~~	16	28	43	104	~~43~~
POSITIVE CONTROL	624	528	1450	1425	822	622
0.001	15	14	23	39	112	57
0.01	16	11	23	38	126	44
0.1	16	7	22	34	125	47
1.0	21	7	27	34	126	49
5.0	12	10	23	43	102	69
+S9 MIX	~~14~~	20	22	~~35~~	161	~~122~~
SOLVENT CONTROL	24	~~24~~	~~20~~	46	~~137~~	142
POSITIVE CONTROL	422	312	1193	1240	727	180
0.001	18	28	21	47	161	140
0.01	21	26	29	38	196	149
0.1	22	21	24	45	173	172
1.0	22	25	20	51	186	142
5.0	23	19	21	60	129	181

*Try+ convertants per plate TEST DATE mo.: 1 day: 10 yr.: 78 by: [illegible]

EVALUATION/CONCLUSION SUBMITTED BY:

Negative [illegible] 3/21

Solvent controls closest to the test values and within the normal spontaneous range were selected. The other value was crossed out.

IBI COMMERCIAL PLATE ASSAY REPORT FORM

CLIENT CIBA-GEIGY CHEMICAL NAME OR CODE NO. Pa-60

DATE RECEIVED mo.: 1 day: 09 yr.: 1978 by: JC ASSAY NO. 2723

TEST CONDITIONS

PHYSICAL DESCRIPTION White solid SOLVENT DMSO

ACTIVATION SYSTEM EMPLOYED IBL

OTHER CONSIDERATIONS

TEST RESULTS

CONCENTRATION — INDICATOR ORGANISMS (REVERTANTS/PLATE)

µg /PLATE	TA-1535	TA-1537	TA-1538	TA-98	TA-100	D6*
-S9 MIX	30	14	20	38	~~107~~	43
SOLVENT CONTROL	~~17~~	~~20~~	~~28~~	~~35~~	136	~~22~~
POSITIVE CONTROL	2115	519	2065	1777	2491	832
0.1	68	10	20	40	83	50
1	53	9	16	39	120	45
10	63	12	13	35	115	52
100	126	16	18	28	142	67
500	250	8	13	30	174	68
+S9 MIX	~~27~~	~~4~~	~~17~~	~~27~~	~~121~~	~~134~~
SOLVENT CONTROL	30	8	17	38	251	157
POSITIVE CONTROL	302	271	1029	801	1119	190
0.1	30	4	17	36	246	159
1	44	6	17	36	270	125
10	45	8	20	40	243	181
100	59	10	17	33	239	163
500	104	8	20	36	319	131
1000	96					

*Tg+ revertants per plate TEST DATE mo.: 1 day: 12 yr: 1978 by:

EVALUATION/CONCLUSION SUBMITTED BY:

Positive to TA1535
Act / NA DNS 4/12

* Solvent controls closest to the test values and within the normal spontaneous range were selected. The other value was circled c.l.

IBI-0184 2-78

LBI COMMERCIAL PLATE ASSAY REPORT FORM

CLIENT NIEH-CEIGY CHEMICAL NAME OR CODE NO. Ma-66

DATE RECEIVED mo.: 4 day: 21 yr.: 78 by: JC ASSAY NO. 3038

TEST CONDITIONS

PHYSICAL DESCRIPTION [illegible] SOLVENT [illegible]

ACTIVATION SYSTEM EMPLOYED [illegible]

OTHER CONSIDERATIONS [illegible]

TEST RESULTS

CONCENTRATION — INDICATOR ORGANISMS (REVERTANTS/PLATE)

/PLATE	TA-1535	CDDV5/30	CDDV5/30	TA-1538	TA-98	TA-100	CDDV5/30
-S9 MIX	12	11	7	25 23	19	28	
SOLVENT CONTROL	19	14	8	38	84	32	
POSITIVE CONTROL	2613	408	1764	1295	1246	409	
.01	19	16	6	20	124	39	
.1	33	16	7	20	179	43	
1	147	14	5	36	673	64	
5	56	3	6	25	524	126	
10	42	2	5	0	357	140	
+S9 MIX	21	11	21	25 42	111	33	
SOLVENT CONTROL	24	23	19	28 43	120	39	
POSITIVE CONTROL	152	456	1139	299 435	749	31	
.01	31	21	16	191 43	165	41	
.1	131	24	10	50 42	313	112	
1	149	15	16	41 45	314	43	
5	207	9	10	96 42	993	127	
10	148	10	2	45 42	347	111	

Try* concentrations per plate TEST DATE mo.: 5,6 day: 11,8 yr: [illegible] by: [illegible]

[illegible] CDDV5/30 CDDV5/30 [illegible] CDDV5/30

EVALUATION/CONCLUSION SUBMITTED BY: [illegible]

[illegible]

[illegible] Bmid Cmid DV in

[illegible] Ach Cmid NM

[illegible] 6/28

* Solvent controls closest to the test series and within the normal spontaneous range were selected, the other value was crossed out.

LBI COMMERCIAL PLATE ASSAY REPORT FORM

CLIENT CIBA-GEIGY CHEMICAL NAME OR CODE NO. Ma-67

DATE RECEIVED mo.: 4 day: 14 yr.: 78 by: JC ASSAY NO. 3089

TEST CONDITIONS

PHYSICAL DESCRIPTION [illegible] SOLVENT [illegible]

ACTIVATION SYSTEM EMPLOYED [illegible]

OTHER CONSIDERATIONS

TEST RESULTS

CONCENTRATION	INDICATOR ORGANISMS (REVERTANTS/PLATE)					
	CDDV 6/5	CDDV 5/30	CDDV 5/30	D.B. mw 5/31	D.B. mw 5/31	CDDV 5/30
Log /PLATE	TA-1535	TA-1537	TA-1538	TA-98	TA-100	D1*
-S9 MIX	12	14	7	~~25~~ 27	89	~~28~~
SOLVENT CONTROL	~~19~~	~~14~~	~~8~~	~~89~~	~~89~~	32
POSITIVE CONTROL	2613	408	1764	1295	1249	409
1	18	10	4	38	93	34
10	18	12	9	45	89	31
100	17	7	18	59	91	27
500	21	13	19	54	121	19
1000	17	10	23	30	121	33
+S9 MIX	21	11	~~11~~	[illegible]	[illegible]	~~33~~
SOLVENT CONTROL	~~14~~	~~23~~	19	[illegible]	[illegible]	39
POSITIVE CONTROL	429	456	1139	759 1357	784	31
1	33	24	9	[illegible]	101	31
10	33	26	11	[illegible]	117	25
100	30	40	132	[illegible]	122	47
500	35	69	356	365 [illegible]	155 [illegible]	47
1000	12	218	396	183 [illegible]	135 [illegible] 185	31

Try convertants per plate TEST DATE mo.: [illegible] day: [illegible] yr: [illegible] by: [illegible]

CDDV 6/5 CDDV 5/30 CDDV 5/30 D.B. mw 5/31 D.B. mw 5/31 CDDV 5/30

EVALUATION/CONCLUSION SUBMITTED BY:

wrong [illegible] for [illegible]
[illegible]

Posted to [illegible] 99
[illegible] 6/28

*Solvent controls closest to the test values and within the normal spontaneous range were selected. The other value was crossed out.

LBI 0184 2/78

CD 5/103/91

ID# 11011

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Basle, Switzerland

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S.H.+E
11-30-81

ARALDITE 0510

SISTER CHROMATID EXCHANGE STUDY

TK 12 759

Chinese Hamster

(Test for mutagenic effects on bone marrow cells)

CG Epoxy Resin 0510

GU 2.3
November 17, 1981

PERMANENT RECORD FILE
TOXICOLOGY

Sister Chromatid Exchange-Study in Somatic Cells, Bone Marrow
TK 12 759, Chinese Hamster
Dosage: 228, 456, 912 mg/kg

No. of experiment: 800653
Batch No.: T-60011
Route: oral

SUMMARY AND ASSESSMENT

TK 12 759 was administered by gavage. Treatment consisted of a single dose. The animals were sacrificed 24 h after the application and 2 h after an intraperitoneal injection of colcemide (10 mg/kg). From the bone marrow drop-preparations were made and stained according to a modified fluorochrome plus Giemsa technique[1,2,3].

The experiments were performed in order to detect a possible mutagenic property of the substance, manifested in somatic cells in vivo in the form of sister chromatid exchanges (SCE)[4].

The numbers of SCE's in the animals treated with all three doses of TK 12 759 were significantly greater than in the concurrent negative controls. A non-parametric test for a dose-related trend likewise yielded a positive result.

A "positive control" experiment with DMBA (100 mg/kg) yielded a mean value of 9.31 SCE's per cell. This is again significantly different from the controls treated with the vehicle (PEG 400) alone.

The results obtained indicate that under the given experimental conditions TK 12 759 provokes an effect that must be interpreted as suggestive of a mutagenic property.

Study director: (Dr. G. Hool)

Date: November 17, 1981

Report reviewed and approved by: (Prof.Dr.D.Müller)

(Head of Experimental Pathology)

Date: November 17, 1981

CIBA-GEIGY Limited

Basle, Switzerland

Protection of Health and Environment

Toxicology

PROCEDURE

1. Data on the animals used.

Chinese hamsters (Cricetulus griseus) of either sex (♀:♂ = 1:1) (weight ♀♀ 21-29 g, ♂♂ 20-28 g) were used. Standard diet: NAFAG No.924. Tap water ad libitum. The animals were kept in an air-conditioned room at a temperature of 21-23°C and a relative humidity of 38-42%. The room was illuminated for 12 hours daily. The animals were identified by individual caging.

2. Treatment schedule.

The treatment groups and the control groups consisted of four female and four male animals each. The animals were treated orally by a single dose of the test material two hours after subcutaneous implantation of a 45 mg-tablet of 5-bromodeoxyuridine (BUdR) (Fa. Heinrich Mack, D-Illertissen, Germany) in the neck. 24 hours after the administration of the test material and 2 hours after intraperitoneal injection of 10 mg colcemide/kg the animals were sacrificed by dislocation of the cervical vertebrae.

a) TK 12 759: 228, 456 and 912 mg/kg in 20 ml/kg Polyethylene Glycol 400 (PEG 400). *)
b) 7,12-Dimethylbenz(a)anthracene (DMBA): 100 mg/kg in 20 ml/kg 0.5% Sodium-Carboxymethylcellulose (CMC), oral application.
Manufacturer of CMC: Hercules Comp., USA.
c) 20 ml/kg PEG 400 (negative control).

3. Preparation of the bone marrow.

Bone marrow from the shafts of both femurs was suspended in balanced salt solution and diluted to hypotonicity with distilled water, kept in a waterbath at 4 to 6°C for 23 min and then centrifuged for 10 min at 200 x g. The pellets were then fixed in methanol-acetic acid 3:1 for a period of

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*) The oral LD_{50} was found to be 2739 (2276-3429) mg/kg in Chinese hamster of either sex (cf. Lab.Report: GU 2.1, dated August 11, 1980).

30 min, resuspended, centrifuged for 5 min at 150 x g, and stored in fresh fixative overnight at 4°C. Finally the pellets again were centrifuged for 5 min at 150 x g, and resuspended in some 0.5 ml fixative in order to obtain a more concentrated cell suspension.

These specimens were pipetted onto wet slides and air-dried.

The air-dried slides then were treated with a solution of bisbenzimide (H 33258) for 15 min, rinsed in McIlvaine-buffer pH 8.0 and irradiated in this buffer at 50°C with UV-light of 350 nm. Following the development of the fluorochrome-UV-light reaction in 60°C 2 x SSC (standard sodium citrate) for 90 min, the slides were stained in 4% Giemsa for 20-40 min, well rinsed, cleared in Xylol and mounted in Eukitt.

4. Scoring of the slides.

The slides of two female and two male animals each of the treatment groups and of the control groups were examined. Twenty-five differently stained metaphases of the second cell-cycle with BUdR-substitution were analysed per animal for the number of SCE's following specific criteria (lit.5).

5. Statistics.

The significance of differences of the treatment groups compared to the negative control group was assessed by t-test on the level of one percent.

As a non-parametric test for a dose-dependent trend on the numbers of SCE's per cell the JONCKHEERE test (lit.6) was used.

RESULTS
(Table 1)

In the intermediate-dose group one female animal died after the administration of colcemide.

In all three groups treated with the various doses of TK 12 759 a statistically highly significant increase in the number of SCE's was found by comparison with the negative controls. The increase in the numbers of SCE's was dose-related, and statistical analysis of the group medians (JONCKHEERE trend test) likewise demonstrated a significant, dose-dependent increase ($p<0.05$, one-sided).

The positive control group showed a highly significant increase of SCE's per cell (9.31) in comparison with the negative control (4.40 SCE's/cell).

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Mutation Res. 56, 169-176 (1978).

[6]JONCKHEERE, A.R.: A distribution-free k-sample test against ordered alternatives.
Biometrika 41, 133-145 (1954).

Table 1 No. of experiment: 800653

THE EFFECT OF TK 12 759 AND 7,12-DMBA ON BONE MARROW CELLS OF CHINESE HAMSTER

Group	No of animals	Sex	Statistics: SCEs per cell — Animal $\bar{x}$ (s)	Group $\bar{x}$ (s)	Observed t value
Control (PEG 400)	1	♀	4.44 (2.47)		
	2	♀	4.80 (2.53)		
	3	♂	4.32 (1.95)		
	4	♂	4.04 (1.93)	4.40 (2.22)	---
DMBA (100 mg/kg) (positive control)	1	♀	8.68 (4.28)		
	2	♀	8.32 (4.36)		
	3	♂	11.20 (6.34)		
	4	♂	8.84 (4.74)	9.31 (5.05)	8.90*
TK 12 759 (228 mg/kg)	1	♀	6.44 (3.18)		
	2	♀	5.88 (2.73)		
	3	♂	6.36 (2.51)		
	4	♂	6.40 (2.99)	6.27 (2.83)	5.20*
TK 12 759 (456 mg/kg)	1	♀	9.12 (3.30)		
	2	♀	7.32 (2.81)		
	3	♂	8.60 (2.74)		
	4	♂	7.96 (3.69)	8.25 (3.18)	9.93*
TK 12 759 (912 mg/kg)	1	♀	14.36 (3.63)		
	2	♀	15.08 (4.66)		
	3	♂	10.96 (3.52)		
	4	♂	11.08 (3.68)	12.87 (4.28)	17.57*

$\bar{x}$ = mean value of SCEs per cell

s = standard deviation

* = statistically significant at 1% (critical t-value = 2.34, d.f. = 198)

JONCKHEERE trend-test likewise positive ($p<0.05$)

Verteiler:

HH. Dr. G. Hool (1x)
Prof.D.Müller (2x)
Dr. A. von Schulthess (2x)

CO 5/103/90

ID# 11013

2

CIBA-GEIGY Limited
Basle, Switzerland

ARALDITE 0510

NUCLEUS ANOMALY TEST IN SOMATIC INTERPHASE NUCLEI

TK 12 759

Chinese Hamster

(Test for mutagenic effects on bone marrow cells)

c H Epoxy Resin 05010

PERMANENT RECORD FILE
TOXICOLOGY

GU 2.3
December 8, 1981

3

Nucleus Anomaly Test
in Somatic Interphase Nuclei
TK 12 759, Chin. Hamster
Dosage: 228, 456, 912 mg/kg

No. of experiment: 800652
Batch: T-60011
Route: oral

SUMMARY AND ASSESSMENT

TK 12 759 was administered by gavage. Treatment consisted of one daily dose of 228, 456 or 912 mg/kg on two consecutive days. The animals were sacrificed 24 h after the second application. From the bone marrow smears were made.

The experiment was performed to evaluate any mutagenic effect on somatic interphase cells in vivo. Mutagenic effects present themselves in interphase cells in form of nucleus anomalies of bone marrow cells[1,2]. These anomalies occur in interphase cells as a consequence of damage during the mitotic process. The increase in anomalies shows a clear dose dependency, comparable to the occurrence of chromosome aberrations in metaphase preparations[3].

The percentages of cells displaying anomalies of the nuclei were significantly greater in the animals treated with all three doses of TK 12 759 than in the concurrent negative controls. The increase was dose-related. A non-parametric test for a dose-related trend likewise yielded a positive result.

A "positive control" experiment with cyclophosphamide (128 mg/kg) yielded 13.8% cells with anomalies of the nuclei. This is again significantly different from the controls treated with the vehicle (PEG 400) alone.

4

These results consequently indicate that oral treatment with TK 12 759 exerted a mutagenic action on somatic cells of the Chinese hamster under the given experimental conditions.

Study director: (Dr. G. Hoel)
Date: December 8, 1981

Responsible for investigation of the slides: (Dr. M. Langauer)
Date: December 8, 1981

Report reviewed and approved by: (Prof.Dr.D.Müller)
(Head of Experimental Pathology)
Date: December 8, 1981

CIBA-GEIGY Limited
Basle, Switzerland
Protection of Health and Environment
Toxicology

PROCEDURE

1. Data on the animals used.

Chinese hamsters (Cricetulus griseus) of either sex (♀:♂ = 1:1)(weight ♀♀ 20-27 g, ♂♂ 22-33 g) were used. Standard diet: NAFAG No.924. Tap water ad libitum. The animals were kept in an air-conditioned room at a temperature of 19-21°C and a relative humidity of 69-78%. The room was illuminated for 12 hours daily. Identification of the animals by individual caging.

2. Treatment schedule.

The preparation was administered orally to groups of 6 female and 6 male animals each. Treatment consisted of daily one application on 2 consecutive days. 24 h after the second application the animals were sacrificed by dislocation of the cervical vertebrae.

a) TK 12 759: 228, 456 and 912 mg/kg in 20 ml/kg Polyethylene Glycol 400 (PEG 400). *)

b) Cyclophosphamide (ENDOXAN®): 128 mg/kg in 20 ml/kg PEG 400 (positive control).
Manufacturer of ENDOXAN®: ASTA-Werke, Germany.

c) 20 ml PEG 400/kg (negative control).

3. Preparation of bone marrow.

Bone marrow was harvested from the shafts of both femurs. In a siliconized pipette filled with approx. 0.5 µl rat serum the bone marrow was drawn up. In order to receive a homogeneous suspension the content of pipette was aspirated gently about three times. Small drops of the mixture were transferred on the end of a slide, spread out by pulling it behind a polished

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*) The oral LD_{50} was found to be 2739 (2276-3429) mg/kg in Chinese hamsters of either sex (cf. Lab.Report: GU 2.1, dated August 11, 1980).

cover glass and the preparations were air-dried. Three hours later, the slides were stained in undiluted May-Grünwald solution for 2 min then in May-Grünwald solution/water 1/1 for 2 min and then in Giemsa's, 40% for 20 min. After being rinsed in methanol 55% for 5-8 sec and washed off twice in water, they were left immersed in water for approx. 2 min. After rinsing with distilled water and air-drying, the slides were cleared in Xylol and mounted in Eukitt.

4. Scoring of the slides.
The slides of three female and three male animals each of the negative control group, the positive control group and of the groups treated with various doses of TK 12 759 were examined. 1000 bone marrow cells each were scored per animal and the following anomalies were registered:
a) Single Jolly bodies, b) fragments of nuclei in erythrocytes, c) micronuclei in erythroblasts, d) micronuclei in leucopoietic cells, e) polyploid cells.

5. Statistics.
The significance of difference was assessed by χ^2-test. As a non-parametric test for a dose-dependent trend on the numbers of cells displaying anomalies of the nuclei the JONCKHEERE test[1] was used.

7

RESULTS
(Table 1)

In the negative control group one female animal died after the second application. In the intermediate-dose group one female died after the first and another after the second application. In the high-dose group two male animals died, one after the first and the other after the second application.

In the negative control the mean percentage of cells displaying anomalies of the nuclei was 0.10. In all three groups treated with the various doses of TK 12 759 the percentage of cells with anomalies was elevated; the low-dose and the intermediate-dose group yielded a mean percentage of 0.90 and the high-dose group of 1.55. Statistical analysis of the values for all three treated groups in relation to the negative control group showed a significant difference at the 5% level. The increase in the percentage of cells displaying anomalies of the nuclei was dose-related, and statistical analysis of the group medians (JONCKHEERE test) likewise demonstrated a significant, dose-dependent increase ($p<0.05$).

The positive control (cyclophosphamide, 128 mg/kg) likewise yielded a marked increase in the percentage of cells with anomalies. Here the mean percentage of anomalies was 13.8. The difference is highly significant ($p<0.05$).

RESULTS
(Table 1)

In the negative control group one female animal died after the second application. In the intermediate-dose group one female died after the first and another after the second application. In the high-dose group two male animals died, one after the first and the other after the second application.

In the negative control the mean percentage of cells displaying anomalies of the nuclei was 0.10. In all three groups treated with the various doses of TK 12 750 the percentage of cells with anomalies was elevated; the low-dose and the intermediate-dose group yielded a mean percentage of 0.90 and the high-dose group of 1.55. Statistical analysis of the values for all three treated groups in relation to the negative control group showed a significant difference at the 5% level. The increase in the percentage of cells displaying anomalies of the nuclei was dose-related, and statistical analysis of the group medians (JONCKHEERE test) likewise demonstrated a significant, dose-dependent increase ($p<0.05$).

The positive control (cyclophosphamide, 128 mg/kg) likewise yielded a marked increase in the percentage of cells with anomalies. Here the mean percentage of anomalies was 13.8. The difference is highly significant ($p<0.05$).

REFERENCES

[1] DOLLER, K. and W. SCHMID (1970), Chemische Mutagenese beim Säuger. Das Knochenmark des Chinesischen Hamsters als in vivo-Testsystem. Hämatologische Befunde nach Behandlung mit Trenimon. Humangenetik 11, 35-54.

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Table 1 Test No.: 800652

THE EFFECT OF TK 12 759 AND CYCLOPHOSPHAMIDE ON BONE MARROW CELLS OF CHINESE HAMSTER

Animals sacrificed 24 h after the second application

Percent of cells with anomalies of nuclei

	Number of animals	Sex of animals	Single Jolly bodies	Fragments of nuclei in erythrocytes	Micronuclei in erythroblasts	Micronuclei in leuco-poietic cells	Polyploid cells	Total
Control (PEG 400)	1	♀	0.1					0.1
	2	♀	0.1					0.1
	3	♀	0.2					0.2
	4	♂	0.1					0.1
	5	♂						0.0
	6	♂	0.1					0.1
Cyclophosphamide (128 mg/kg)	1	♀	11.0	1.5	0.9	0.4		13.8
	2	♀	8.3	1.7	2.2	0.9	0.1	13.2
	3	♀	10.1	1.6	1.6	1.1		14.4
	4	♂	9.0	0.4	1.5	0.4		17.1
	5	♂	10.5	1.9	1.2	0.2		13.8
	6	♂	10.6	2.2	1.1	1.3		15.2
TK 12 759 (228 mg/kg)	1	♀	0.6					0.6
	2	♀	0.6					0.6
	3	♀	0.8	0.1	0.1	0.2		1.2
	4	♂	0.5			0.1		0.6
	5	♂	1.2	0.1	0.1			1.4
	6	♂	0.4		0.4	0.2		1.0
TK 12 759 (456 mg/kg)	1	♀				0.2		0.2
	2	♀	0.4	0.1	0.1	0.3		0.9
	3	♀	1.0		0.2	0.1		1.3
	4	♂	0.9					0.9
	5	♂	1.4			0.2		1.6
	6	♂	0.4		0.1			0.5
TK 12 759 (912 mg/kg)	1	♀	1.5			0.2		1.7
	2	♀	1.0		0.1			1.1
	3	♀	0.3					0.3
	4	♂	2.4			0.1		2.5
	5	♂	1.7					1.7
	6	♂	1.9		0.1			2.0

10

Verteiler:

Herrn Dr. C. Hool	(1x)
Frau Dr. M. Langauer	(1x)
Herrn Prof.D.Müller	(2x)
Herrn Dr. A. von Schulthess	(2x)

CO 5/10/98

I.D. # 16607

Epoxy 0510

CIBA-GEIGY LIMITED

BASLE, SWITZERLAND

Test No.: 874067

Protection of Health
and Environment

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PRODUCT SAFETY,
AND ECOLOGY

GU 7
Genetic Toxicology

R E P O R T

MAMMALIAN SPOT TEST, MOUSE, 8 WEEKS

Test material: TK 12 759 (EPOXY 0510)

Date: October 20, 1987

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I N D E X

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Procedure	Page 7
Results	Page 10
References	Page 11

GENERAL

Type of study:	Mammalian Spot Test, Mouse, 8 Weeks
Animal species:	Male mice: T-stock Female mice: C57 Bl/6J
Origin:	Bomholtgard Ltd., Denmark
Purpose:	Detection of genetic effects of several kinds on somatic cells, including point mutations, recombinations and deletions (through breakage or nondisjunction).
Test material:	TK 12 759 (EPOXY 0510)
Batch No.:	T-60011
Purity:	Commercial grade
Validity:	Information provided by sponsor on request
Stability:	Stable (ensured by sponsor)
Sponsor:	CIBA-GEIGY Limited, Plastics and Additives Division, Basle, Switzerland
Testing facility:	CIBA-GEIGY Limited, Basle, Switzerland, GU 7, Genetic Toxicology. Laboratories and Animals facilities of Genetic Toxicology, R-1066, GU 7.
Personnel:	Study Director: Mr. F. Strasser Technical conduct: Mr. H. Loos, Mr. Ph. Rapp Statistics: Mr. A. Grieve (FO 5.31)
Test No.:	874067
SOP No.:	300107

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Route of administration:	Intraperitoneal
Dosage:	Tolerability test: First step : 200.0, 1000.0 and 5000.0 mg/kg Second step: 120.0 mg/kg Mutagenicity test: 30.0, 60.0 and 120.0 mg/kg
Vehicle:	Sesame oil
No. of animals per group:	In the tolerability test: 4 females, In the mutagenicity test: 96 females and 48 males in the treatment groups and the negative and positive control groups.
Starting date (first administration):	April 8, 1987
Date of study completion:	August 13, 1987
Reported and filed:	October 1987
Location of archives:	CIBA-GEIGY Limited, Basle, Switzerland, Archives of Genetic Toxicology, GU 7.

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STUDY TITLE :

TK 12759 (EPOXY 0510)
MAMMALIAN SPOT TEST, 8 WEEKS

Sponsor : KA/AD
Test No. : 874067
Study director : STRASSER F.

QAU Name : BALTISBERGER B.

THE QUALITY ASSURANCE INSPECTION(S) WAS (WERE) PERFORMED ON THE FOLLOWING DATE(S)

1 16.03.87
2 13.05.87
3 25.05.87
4 15.10.87 FINAL REPORT AUDIT

THE FINDINGS WERE REPORTED TO THE STUDY DIRECTOR AND TO THE MANAGEMENT ON THE FOLLOWING DATE(S):

1 16.03.87
2 14.05.87
3 25.05.87
4 15 10.87

QSE/QAU Manager : Dr. H. J. Weidels

i.A. W. Morgenthaler 15.10.87

Signature Date

Distribution : 0 Study director 0 GU 1.1 File

SUMMARY AND ASSESSMENT

TK 12 759 (EPOXY 0510) was administered in a single intraperitoneal injection to pregnant female mice (C57 Bl/6J) on the 10th day after conception. Doses of 30.0, 60.0 and 120.0 mg/kg were given.

This test system is designed to ascertain whether the substance might have a mutagenic effect on somatic cells in vivo. The test system permits the detection of induced point mutations and other genetic events in the melanoblasts of embryos exposed in utero. The induction of mutation is monitored post-natally by examination of the coat of young mice for recessive spots (RS) resulting from expression of recessive genes involved in coat-colour determination (Refs. 1, 2, 3).

In the experiment performed, the average litter size was not markedly affected by any of the doses administered. The survival of the young animals between birth and the age of observation for spots (approx. 12 days) was also not affected by any of the doses applied. 226 offspring of the negative control, 324 of the positive control and 349, 290 and 292 animals of the doses 30.0, 60.0 and 120.0 mg/kg respectively were examined for spots. 0.89% of the control animals showed RS. The frequencies of RS in the group treated with 30.0, 60.0 and 120.0 mg/kg were respectively 0.57, 0.69 and 0%. Thus, the relative incidence of RS among the animals treated with the various doses of TK 12 759 (EPOXY 0510) did not differ significantly ($p>0.05$) from that of the control (sesame oil).

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By contrast, the "positive control" experiment with ethylnitrosourea (50.0 mg/kg) performed simultaneously yielded an average RS frequency of 6.17%. This is significantly different (Fisher's exact test, for RS $p = < 0.0014$) from the negative control (0.89%) and is in accordance with the positive control data derived from the experiment of February 1986 with the doses of 25.0, 50.0 and 75.0 mg/kg. Overall test: $X^2(3) = 42.00$, $p< 0.0001$. Trend test: $X^2(1) = 31.83$, $p< 0.0001$, $Z = 5.2086$ (one sided, $p< 0.0001$) (Table 2).

It is concluded that, under the given experimental conditions, no evidence of mutagenic effects was obtained in the offspring of pregnant mice treated with TK 12 759 (EPOXY 0510).

Study Director:

(F. Strasser)

Date: October 19, 1987

Report reviewed and approved by:

(Dr. P. Arni)

(Senior Scientist, Genetic Toxicology)

Date: October 20, 1987

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PROCEDURE

1. Data on the animals used

Mature mice (females = C 57 Bl/6J , males = T-stock) female:male = 2:1) about 3-4 months old were obtained from Bomholtgard Ltd. Denmark. The acclimatization time was more than a week. The body-weight of the females was 21-27 g (tolerability test) and 19-32 g (mutagenicity test); the body weight of the males was not determined. The animals were fed a standard diet of NAFAG No. 890 pellets with tap water ad libitum and were kept in an air-conditioned room at a temperature of 21-23°C and a relative humidity of 45-62%, illuminated for 12 hours daily. The females animals were separated and randomized. After successful mating the animals were identified by individual caging.

2. Tolerability test

A preliminary test was performed to determine the highest dosage of the test substance to be applied in the mutagenicity assay.

In a tolerability test three groups of four female mice are treated with three different doses, one receiving the maximum dose of 5000 mg/kg, or the highest applicable dose, and the other two doses of 1/5 and 1/25 of that amount respectively. The animals are treated with a single dose. The observation period lasts two weeks. If all animals in all dose groups die in the first test, a second is performed in which the highest dose given is 3/4 of the lowest used in the preceding test. If some of the animals in one of the dose groups die, the test is continued with a high dose corresponding to a predetermined fraction of that dose. Depending on the outcome, the highest dose causing no deaths is used as the highest in the mutagenicity test, or if necessary the test is repeated with lower doses.

In the tolerability test performed on non-pregnant female mice the dose of 120.0 mg/kg was found to be suited as the highest in the mutagenicity assay and was administered, together with a further two doses, diminishing by a factor of 0.5.

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3. Mating schedule in the mutagenicity test

Each group consists of 48 male and 96 female animals. One untreated male is placed in a cage with 2 untreated females. The females are examined daily for successful mating, as indicated by the formation of a vaginal plug. The day on which a vaginal plug is observed is designated as " day 1/2 of gestation". Each day, all females presumed to be pregnant are removed. The procedure is continued for four consecutive days (4 mating nights). Subsequently, the presumably pregnant female animals are uniformly distributed among the respective groups by random selection.

4. Treatment schedule

The preparation was administered intraperitoneally to groups of 72 successfully mated females. All presumably pregnant females were treated on the 10th day after conception. Treatment consisted of a single intraperitoneal injection of the respective dose. The female animals of the control group received the vehicle only.

a) TK 12 759:
30.0, 60.0 and 120.0 mg/kg in 10 ml/kg sesame oil

b) 10 ml/kg sesame oil (negative control).
Manufacturer of sesame oil: Siegfried AG, Switzerland

c) N-Nitroso-N-ethylurea (ENU) (positive control): 50 mg/kg in 20 ml/kg Hank's BSS
Manufacturer of ENU: Sigma Comp., USA.
Manufacturer of Hank's balanced salt solution: Gibco AG, Switzerland.

5. Positive control

Data from the positive control group with N-Nitroso-N-ethylurea (ENU), 50.0 mg/kg in 20 ml/kg Hank's BSS (i.p.), carried out in parallel, are shown in Table 1 and data from an ENU experiment with three doses (25.0, 50.0 and 75.0 mg/kg) performed during February 1986 are shown in Table 2.

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6. Examination of offspring

Animals treated as embryos are allowed to come to birth. The numbers of live and dead offspring are listed. They are inspected for external visible morphological changes. The examination upon spots begins at the age of 12 -14 days and is carried out twice per week during three weeks. Two classes of spots are distinguished and registered: (a) pigmented and white spots (recessive spots, RS) randomly distributed on the coat which are presumed to result from recessive mutations and (b) white mid-ventral spots (WMVS) within 5 mm of the mid-ventral line presumably arising from cell killing and thus not a result of mutagenic effects. Yellow, agouti-like, spots in the vicinity of the mammae, genitalia, throat, axillary and inguinal areas and on the mid-forehead, which are presumed to result from misdifferentiation (MDS) are omitted from scoring. The pelts from the animals with spots are preserved.

7. Statistics

The statistical analysis is performed in two parts. Firstly, the numbers of recessive spots in the control group and in the treatment groups are compared by a chi-squared test.

Secondly, a test is carried out to determine whether the frequency of recessive spots increases with increasing doses. Such tests are admissible within the framework of generalized linear models (Ref.4). An alternative rationale of the trend test is to be found in Tarone and Gart, 1980 (Ref.5).

In case of a dose-related effect of a substance, the trend test is preferable, since it provides a more sensitive rejection of the null hypothesis of equal proportions. In addition the overall test does not distinguish between an increase or decrease with respect to the control proportion.

The tests are applied on the condition that the proportions of recessive spots are constant over litters. Experience to date shows that this assumption cannot be rejected largely because the proportions of recessive spots are very low.

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RESULTS

(Table 1)

In the tolerability test the dose of 120.0 mg/kg of TK 12 759 (EPOXY 0510) proved to be the highest causing no deaths in a group of four animals. Therefore, this dose was selected as the highest in the mutagenicity test.

From 72 presumably pregnant females per dose group, the following numbers actually were pregnant and gave birth to litters: control: 41 , 30.0 mg/kg: 50 , 60.0 mg/kg: 43 and 120.0 mg/kg: 46. The average litter sizes registered were: control, 6.10; treatment groups 7.04, 7.21 and 6.70, respectively. The results of the matings are given in Table 1.

226 offspring from the control group and 349, 290 and 292 animals from the groups treated with 30.0, 60.0 and 120.0 mg/kg, respectively, were examined for colour spots. The following percentages of animals with recessive (RS) and white mid-ventral spots (WMVS) were recorded from gross observations:

a) RS

0.89% (Control), 0.57% (30.0 mg/kg), 0.69% (60.0 mg/kg), 0% (120.0 mg/kg).

Statistical analysis for RS

Overall test: $X^2(3) = 3.690$, $p = 0.2969$
Trend test : $X^2(1) = 1.702$, $p = 0.1920$ (one-sided, $p = 0.9040$)

b) WMVS

0% (Control), 0.86% (30.0 mg/kg), 1.38% (60.0 mg/kg), 1.37% (120.0 mg/kg).

In the positive control (N-Nitroso-N-Ethylurea, 50.0 mg/kg) the mean percentage of RS spots was 6.17.
Fisher's exact test : $p = < 0.0014$.

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in A. Hollaender and F.J. de Serres (Eds.), Chemical Mutagens, Principles and Methods for Their Detection, Plenum, New York, 151-176.

[2]Russell, L.B., Selby, P.B., Von Halle, E., Sheridan and L. Valcovic (1981), Use of the Mouse Spot Test in Chemical Mutagenesis Interpretation of Past Data and Recommendations for Future Work.
Mutation Res. 86, 355-379.

[3]Gocke, E., Wild, D., Eckhardt, K. and M.-T. King (1983), Mutagenicity Studies with the Mouse Spot Test.
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Table 1

No. of experiment: 874067

THE EFFECT OF TK 12 759 ON OFFSPRING FROM C57 Bl/6J x T CROSS TREATED IN UTERO

Dose mg/kg	Vehicle	Route	Treated *females* with vaginal plug	% *females* with litter	Average litter size	NUMBER of O F F S P R I N G examined	with recessive spots		with white mid-ventral spots	
							total	%	total	%
Neg. Contr.	Sesame oil	i.p.	72	56.9	6.10	226	2	0.89	0	0
30	Sesame oil	i.p.	72	69.4	7.04	349	2	0.57	3	0.86
60	Sesame oil	i.p.	72	59.7	7.21	290	2	0.69	4	1.38
120	Sesame oil	i.p.	72	63.9	6.70	292	0	0	4	1.37
Pos. Contr. (ENU 50.0 mg/kg)	Hank's BSS	i.p.	72	72.2	6.81	324	20	6.17	10	3.09

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Table 2 Date of experiment: February 1986

Positive control data / *N-Nitroso-N-Ethylurea (ENU)* 25.0 / 50.0 / 75.0 mg/kg

(ON OFFSPRING FROM C57 Bl/6J x T CROSS TREATED IN UTERO)

Dose mg/kg	Vehicle	Route	Treated females with vaginal *plug*	% females with litter	Average litter size	NUMBER of OFFSPRING examined	with Recessive spots		with White mid-ventral spots	
							total	%	total	%
Contr.	Hank's *BSS*	i.p.	66	68.2	6.58	277	0	0	3	1.08
25.0	Hank's BSS	i.p.	66	74.2	7.37	350	14	4.0	10	2.86
50.0	Hank's BSS	i.p.	66	66.7	7.18	298	20	6.71	5	1.68
75.0	Hank's *BSS*	i.p.	66	71.2	7.21	270	27	10.0	18	6.67

Verteiler:

HH. Dr. A. v. Schulthess	(2x)
Prof. D. Müller	(1x)
F. Strasser	(2x)

I U C L I D

Data Set

Existing Chemical	:	ID: 2210-79-9
CAS No.	:	2210-79-9
EINECS Name	:	2,3-epoxypropyl o-tolyl ether
EC No.	:	218-645-3
Molecular Formula	:	C10H12O2

Producer related part

Company	:	Huntsman (Europe) BVBA (ehemals ICI Polyurethanes)
Creation date	:	13.11.2006

Substance related part

Company	:	Huntsman (Europe) BVBA (ehemals ICI Polyurethanes)
Creation date	:	13.11.2006
Status	:	
Memo	:	
Printing date	:	21.11.2006
Revision date	:	
Date of last update	:	20.11.2006
Number of pages	:	25
Chapter (profile)	:	Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile)	:	Reliability: without reliability, 1, 2, 3, 4
Flags (profile)	:	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1. General Information

Id 2210-79-9
Date 21.11.2006

1.0.1 APPLICANT AND COMPANY INFORMATION

Type	:	importer of product
Name	:	Huntsman Corporation (formerly Vantico)
Contact person	:	Dr. Philip Anderson
Date	:	
Street	:	10003 Woodloch Forest Drive
Town	:	77380 The Woodlands, TX
Country	:	United States
Phone	:	
Telefax	:	
Telex	:	
Cedex	:	
Email	:	Philip_Anderson@huntsman.com
Homepage	:	

20.11.2006

1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR

1.0.3 IDENTITY OF RECIPIENTS

1.0.4 DETAILS ON CATEGORY/TEMPLATE

1.1.0 SUBSTANCE IDENTIFICATION

IUPAC Name	:	oxirane, [(2-methylphenoxy)methyl]
Smiles Code	:	
Molecular formula	:	
Molecular weight	:	
Petrol class	:	

13.11.2006

1.1.1 GENERAL SUBSTANCE INFORMATION

Purity type	:	typical for marketed substance
Substance type	:	organic
Physical status	:	liquid
Purity	:	> 95 % v/v
Colour	:	clear, colorless to slight yellow
Odour	:	

20.11.2006

1.1.2 SPECTRA

Type of spectra	:	IR
Result	:	Identity ascertained by IR

	Epoxid:	5.47 Eq/kg
	Free hydroxyl:	0.50 Eq/kg
	Total chlorine:	0.21 %
	Hydrolyzable chlorine:	242 ug/g
	Ionic chlorine:	< 10 ug/g
	Epichlorhydrin:	75 ug/g
Type of spectra	: GC	
Result	: Gas chromatogram (by area percent)	
	Main product:	95.9 %

1.2 SYNONYMS AND TRADENAMES

cresyl glycidyl ether

20.11.2006

Araldite DY023

20.11.2006

Araldite DY-K

20.11.2006

1.3 IMPURITIES

1.4 ADDITIVES

1.5 TOTAL QUANTITY

1.6.1 LABELLING

1.6.2 CLASSIFICATION

1.6.3 PACKAGING

1.7 USE PATTERN

1.7.1 DETAILED USE PATTERN

1.7.2 METHODS OF MANUFACTURE

1.8 REGULATORY MEASURES

1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES

1.8.2 ACCEPTABLE RESIDUES LEVELS

1.8.3 WATER POLLUTION

1.8.4 MAJOR ACCIDENT HAZARDS

1.8.5 AIR POLLUTION

1.8.6 LISTINGS E.G. CHEMICAL INVENTORIES

1.9.1 DEGRADATION/TRANSFORMATION PRODUCTS

1.9.2 COMPONENTS

1.10 SOURCE OF EXPOSURE

1.11 ADDITIONAL REMARKS

1.12 LAST LITERATURE SEARCH

1.13 REVIEWS

2.1 MELTING POINT

2.2 BOILING POINT

2.3 DENSITY

Type	:	density
Value	:	1.08 g/cm³ at 25 °C
Method	:	
Year	:	
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4

14.11.2006 (1)

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

2.5 PARTITION COEFFICIENT

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

2.6.2 SURFACE TENSION

2.7 FLASH POINT

2.8 AUTO FLAMMABILITY

2.9 FLAMMABILITY

2.10 EXPLOSIVE PROPERTIES

2.11 OXIDIZING PROPERTIES

2.12 DISSOCIATION CONSTANT

2.13 VISCOSITY

2.14 ADDITIONAL REMARKS

3.1.1 PHOTODEGRADATION

3.1.2 STABILITY IN WATER

3.1.3 STABILITY IN SOIL

3.2.1 MONITORING DATA

3.2.2 FIELD STUDIES

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

3.3.2 DISTRIBUTION

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Type	:	aerobic
Inoculum	:	activated sludge, domestic
Concentration	:	10.8 mg/l related to Test substance 20.47 mg/l related to Test substance
Contact time	:	28 day(s)
Degradation	:	3 - 4 (±) % after 28 day(s)
Result	:	under test conditions no biodegradation observed
Kinetic of testsubst.	:	3 day(s) 1 % 8 day(s) 2 % 17 day(s) 3 % 24 day(s) 3 % 28 day(s) 3 %
Control substance	:	Aniline
Kinetic	:	8 day(s) 53 % 28 day(s) 84 %
Deg. product	:	
Method	:	OECD Guide-line 301 B "Ready Biodegradability: Modified Sturm Test (CO2 evolution)"
Year	:	1981
GLP	:	
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	10.8 mg test substance/l = 4% biodegradation in 28 days 20.47 mg test substance/l = 3% biodegradation in 28 days
Test condition	:	Temperature: 22 +/- 2 degree C Air: 25 ml/min free of CO2 Bacteria collected from activated sludge of treatment plant of CH-4153 Reinach on 13/03/89. Reference substance: aniline, 20 mg/l

Due to poor solubility, the test substance was homogenized with nonylphenol before addition to the medium.

Reliability : (1) valid without restriction
Flag : confidential, Critical study for SIDS endpoint
20.11.2006

(2)

3.6 BOD5, COD OR BOD5/COD RATIO

3.7 BIOACCUMULATION

3.8 ADDITIONAL REMARKS

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	static
Species	:	Brachydanio rerio (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC0	:	10
LC50	:	13
LC100	:	18
Limit test	:	
Analytical monitoring	:	no
Method	:	OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year	:	1984
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	LC50 was calculated according to Berkman, JASA 48:569-599. 1953. LC50 Values were graphically determined on gausso-logarithmic probability paper. Values based on nomimal concentrations
Result	:	Blank control = 0% mortality Vehicle control = 0% mortality
Test condition	:	Temperature: 23+/- 1 degree C pH: 7.9 - 8.4 O2: 81-100% Water: dechlorinated tap water Hardness: 184mg CaCO3/l Lighting: fluorescent light 16 hours/day Stock solution: no stock solution was prepared. The test substance and 4 mg alkylphenol-polyglycol ether were added directly to water in the tanks. 10 fishes per concentration and control Concentrations tested: 5.8, 10. 18, 32, and 58 mg/l
Reliability	:	(1) valid without restriction
Flag	:	confidential, Critical study for SIDS endpoint

20.11.2006 (3)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	static
Species	:	Daphnia magna (Crustacea)
Exposure period	:	24 hour(s)
Unit	:	mg/l
EC0	:	10
EC50	:	16
EC100	:	32
Analytical monitoring	:	no
Method	:	OECD Guide-line 202
Year	:	1984
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	EC50 was calculated according to Spearman-Kaerber in: DJ Finney, 524-530. London, 1964. EC50 Values were graphically determined on gausso-logarithmic probability paper. Values based on nomimal concentrations
Result	:	Blank control = 0% immobilization

		Vehicle control = 5% immobilization
Test condition	:	Temperature: 20 +/- 1 degree C pH: 7.6 - 7.8 O2: 93-95% Lighting; 1500 lux for 16 hours/day Stock solution: 100 mg test substance and 4 mg alkylphenol-polyglycol ether mixed and added to water to make 1000ml. 20 Daphnia per concentration (4 replicates of 5 Daphnia each). Concentrations tested; 10, 18, 32, 58, 100 mg/l
Reliability	:	(1) valid without restriction
Flag	:	confidential, Critical study for SIDS endpoint

20.11.2006 (4)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

Type	:	aquatic
Species	:	activated sludge, domestic
Exposure period	:	3 hour(s)
Unit	:	mg/l
IC50	:	> 100
IC20	:	> 100
Analytical monitoring	:	no
Method	:	OECD Guide-line 209 "Activated Sludge, Respiration Inhibition Test"
Year	:	1984
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Inhibitions were calculated on the basis of the oxygen consumption as compared to the blank. Reference substance (3,5-dichlorophenol) IC50 = 15 mg/l
Test condition	:	Temperature: 20 +/- 2 degree C Air: 25 ml/min free of CO2 Water: dechlorinated drinking water Activated sludge collected from sewage treatment plant of CH-4153 Reinach on 20/02/89. The sludge was separated from the aqueous layer by settling instead of centrifugation. Sludge concentration: 1.7g/l Reference substance: 3,5-dichlorophenolat concentrations of 3.2, 10, and 32 mg/l
Reliability	:	(1) valid without restriction
Flag	:	confidential, Critical study for SIDS endpoint

20.11.2006 (5)

4.5.1 CHRONIC TOXICITY TO FISH

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS

4.6.4 TOX. TO OTHER NON MAMM. TERR. SPECIES

4.7 BIOLOGICAL EFFECTS MONITORING

4.8 BIOTRANSFORMATION AND KINETICS

4.9 ADDITIONAL REMARKS

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD50
Value	:	ca. 5800 mg/kg bw
Species	:	rat
Strain	:	other: RAI/f
Sex	:	male/female
Number of animals	:	6
Vehicle	:	other: polyethylene glycol
Doses	:	2150, 3170, and 6000 mg/kg
Method	:	other: similar to Directive 84/449/EEC, B.1
Year	:	
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	Within 2-4 hours of treatment, the animals showed dyspnea, lachrymation, ruffled fur and curved position. The animals had recovered within 5 days. All animals were sacrificed and examined after a 7 day observation period. No animals died at doses of 2150 and 3170 mg/kg bw. At a dose of 6000 mg/kg, 2/5 males and 4/5 females died. Congested livers were noted in the deceased animals. No substance related gross organ changes were seen in the sacrificed animals.
Reliability	:	(2) valid with restrictions
Flag	:	confidential, Critical study for SIDS endpoint

13.11.2006 (6)

5.1.2 ACUTE INHALATION TOXICITY

Type	:	LC50
Value	:	6090 mg/m³
Species	:	rat
Strain	:	other: Tif RAI/f (SPF)
Sex	:	male/female
Number of animals	:	20
Vehicle	:	other: neat
Doses	:	999, 2746, 3973, 8142 mg/m3
Exposure time	:	4 hour(s)
Method	:	other:
Year	:	
GLP	:	
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	Sachsse, Ullman, et al. 1973. Proceedings of the European Society for the study of drug toxicity. Vol.XV. p239-251.
Result	:	Within 2-4 hours of treatment, the animals in all dose levels showed dyspnea, exophthalmos, ruffled fur and curved position. Animals in the highest dose showed ataxy. No animals died at doses of 999 and 2746 mg/m3. 4/10 males died at 3973 mg/m3 and at 8142 mg/m3 9/10 males and 6/10 females died. At a dose of 6000 mg/kg, 2/5 males and 4/5 females died. Small isolated hemorrhages were noted in the lungs of the deceased animals. No substance related gross organ changes were seen in the sacrificed animals.

Test condition	:	Animals were exposed nose only for 4 hours, after which the animals were returned to cages and observed for 14 days.
Reliability	:	(2) valid with restrictions
Flag	:	confidential, Critical study for SIDS endpoint

20.11.2006 (7)

5.1.3 ACUTE DERMAL TOXICITY

Type	:	LD50
Value	:	> 2150 mg/kg bw
Species	:	rat
Strain	:	other: RAI/f
Sex	:	male/female
Number of animals	:	6
Vehicle	:	other: neat
Doses	:	2150 mg/kg bw
Method	:	other: Neakes and Sanderson. 1969. Brit. J. Indust. Med 26:59-64
Year	:	
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	There were no deaths. No symptoms and no local irritation was observed. After a 7 day observation period, the rats were sacrificed and examined. No substance related gross organ changes were seen.
Test condition	:	About 6 hours before treatment, the backs of rats were shaved with an electric razor. The suspension was evenly dispersed on the skin with a syringe and covered with aluminum foil and held in place with "iso-elast" plaster. After 24 hours, the plaster and foil were pealed off and the skin cleaned with warm water to remove all test substance. Due to technical reasons, no higher doses were possible.
Reliability	:	(2) valid with restrictions
Flag	:	confidential, Critical study for SIDS endpoint

20.11.2006 (8)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

5.2.1 SKIN IRRITATION

Species	:	rabbit
Concentration	:	undiluted
Exposure	:	Occlusive
Exposure time	:	24 hour(s)
Number of animals	:	6
Vehicle	:	other: neat
PDII	:	5.2
Result	:	moderately irritating
Classification	:	irritating
Method	:	other: according to the "appraisal of Safety of Chemicals in Foods, Drugs, Cosmetics. US Food and Drug Officials
Year	:	1959
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	The test was peformed on 3 male and 3 female rabbits. The entire back and flank were shaved with electric clippers and immediately before treatment, the skin on the left side was slightly scarified. The test substance was applied to intact and abraded skin, covered with an

impermeable material and fastened with adhesive tape. The dressings were removed after 24 hours and scored for irritation. The skin irritation was scored again 48 hours after removal of the dressing.

Reliability : (2) valid with restrictions
Flag : confidential, Critical study for SIDS endpoint
20.11.2006 (9)

5.2.2 EYE IRRITATION

Species : rabbit
Concentration : undiluted
Dose : .1 other:g
Exposure time : 24 hour(s)
Comment : other: the eyes of 3 rabbits were rinsed with water after 30 seconds; the eyes of 3 rabbits were not rinsed
Number of animals : 6
Vehicle : none
Result : slightly irritating
Classification : not irritating
Method : other: according to the "appraisal of Safety of Chemicals in Foods, Drugs, Cosmetics. US Food and Drug Officials
Year : 1959
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Method : The reactions were appraised with a slit-lamp after 24 hours and 2, 3, 4, and 7 days post treatment.
Result : The irritation score was found to be 0 for the cornea, 0 for the iris, and 0.8 for the conjunctivae, out of a total maximum possible score of 110. The test substance is considered to be a slight irritant to the rabbit eye.
Reliability : (2) valid with restrictions
Flag : confidential, Critical study for SIDS endpoint
20.11.2006 (10)

5.3 SENSITIZATION

Type : Guinea pig maximization test
Species : guinea pig
Concentration : 1st: Induction 3 % intracutaneous
2nd: Induction 10 % occlusive epicutaneous
3rd: Challenge 3 % occlusive epicutaneous
Number of animals : 20
Vehicle : other: sesame oil for intradermal; vaseline for epidermal
Result : sensitizing
Classification : sensitizing
Method : OECD Guide-line 406 "Skin Sensitization"
Year : 1981
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Remark : The test was performed on 10 male and 10 female guinea pigs per group.
1st Induction: 3 intradermal injections (0.1 ml each) were made simultaneously into the shaved neck of guinea pigs as follows: (Fruends adjuvant/saline 1:1) (test article in sesame oil) (test article in adjuvant)
2nd Induction: after one week, the test substance in vaseline was applied on a filterpaper patch to the neck of animals for 48 hours.
Challenge: two weeks aftoer epidermal induction, animals were tested with the test substance in vaseline and vehicle alone on a filter paper applied to

the flank for 24 hours.
Control group: treated with adjuvant and vehicle during induction and vehicle during challenge. Ten control animals were treated with the test substance during challenge to check for irritancy.
A preliminary experiment with 3, 10 and 30% test substance in vaseline demonstrated erythema with 10 and 30%.

Result : Under test conditions, 100% of the animals showed skin reactions at 24 and 48 hours after challenge. TK 10410 is classified as an extreme dermal sensitizer in albino guinea pigs according to the grading of Magnusson and Kligman.

Reliability : (1) valid without restriction
Flag : confidential, Critical study for SIDS endpoint
20.11.2006 (11)

Type : Patch-Test
Species : human
Concentration : 1st: Induction 1 % semiocclusive
2nd:
3rd:
Number of animals : 50
Vehicle : CMC
Result : sensitizing
Classification : sensitizing
Method : other: Human Repeated Insult Patch test
Year :
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Method : A concentration of 1% (v/v) in 1% CMC was used in the main study after conducting a preliminary irritation study.
Due to a delayed raction in the screening study group, the patch applications were terminated in the main study group after the first induction patch. The participants were observed for 72 hours after patch removal.

Result : 5/5 subjects treated with neat test substance in the screening study showed a 4+ reaction through 72 hours and for 4 weeks following the induction application.
1/4 subjects treated with 1% test substance in CMC in the screening study showed a 4+ reaction through 72 hours and for 3 weeks following the induction application.
2/50 subjects treated with 1% test substance in CMC in the main study showed a 4+ reaction through 72 hours and for 2 weeks following the induction application.

Conclusion : Based on the incidence and severity of the reactions, it was concluded that under the conditions of this study, TK 10410 would be a sensitizing agent in humans.

Reliability : (2) valid with restrictions
Flag : confidential, Critical study for SIDS endpoint
20.11.2006 (12)

Type : Maurer optimisation test
Species : guinea pig
Concentration : 1st: Induction .1 % intracutaneous
2nd: Induction .1 % intracutaneous
3rd: Challenge .1 % intracutaneous
Number of animals : 20
Vehicle : physiol. saline
Result : not sensitizing
Classification : not sensitizing
Method : other: Maurer optimisation test
Year : 1975
GLP :

Test substance	:	
Method	:	On day 1 the test substance (0.1%) in saline without adjuvant was injected intradermally at 2 sites (back and flank) and on days 3 and 5 injected at one site on the back. 24 hours later the skin reaction was assessed by measuring the area of erythema and the skin-fold thickness. The 'reaction volume' was calculated from these values for each animal. During weeks 2 and 3, six doses (0.1ml) consisting of the test substance mixed 1:1 with adjuvant was injected into the skin of the neck on Monday, Wednesday and Friday. Two weeks after the last induction dose, 0.1ml of the initial test substance formulation was injected intradermally into the untreated flank. Twenty-four hours later, the 'reaction volume' was assessed. A group of 20 animals were treated in the same manner with vehicle only, and another group of 20 animals were treated with dinitrochlorobenzene as positive controls. A positive was defined as final 'reaction volume' exceeding the mean + 1 Std Dev of the 4 'pre-sensitizing' responses. Statistical significance between controls and treated animals was assessed by the Fisher test.
Result	:	Intradermal injections of vehicle alone failed to induce skin sensitization (0/20). 100% (20/20) of DNCB treated animals were positive reactors. 15% (3/20) of test substance treated animals were positive reactors.
Conclusion	:	TK10410 is considered to possess no skin-sensitizing potential in albino guinea pigs.
Reliability	:	(2) valid with restrictions
Flag	:	confidential, Critical study for SIDS endpoint

20.11.2006 (13)

5.4 REPEATED DOSE TOXICITY

Type	:	Sub-acute
Species	:	rat
Sex	:	male/female
Strain	:	other: RAI f SPF (RA 25)
Route of admin.	:	inhalation
Exposure period	:	21 days
Frequency of treatm.	:	6 hrs/day, 5 days/week
Post exposure period	:	21 days
Doses	:	0, 53 (+/- 4), 152 (+/- 4), and 305 (+/- 10) mg/m3
Control group	:	yes, concurrent vehicle
NOAEL	:	< 53 mg/m³
LOAEL	:	53 - mg/m³
Method	:	Directive 84/449/EEC, B.8 "Sub-acute toxicity (inhalation)"
Year	:	
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	At the highest dose tested, 6 males and 9 females died during the exposure period. There was a significant decrease in body weight and food consumption as compared to controls. Animals had acute congestion and occasional hemorrhaging in the myocardium, lungs, liver, kidney, adrenal, ovaries, and brain. 5 of 10 male rats showed reduced spermatogenesis. The 152 mg/m3 dose showed similar acute effects as well as decreased body weight and food consumption. Only 1 of 10 male rats showed reduced spermatogenesis.

At the lowest dose, 53 mg/m3, the decrease in body weight and food consumption was still statistically significant.

Blood chemistry and hematology values were unremarkable and comparable to the controls

Test substance : Lot No. IP 6.2.90
o-CGE 95.3%
hydrolyzable CGE 2.0%
unknown 1.4%
hydrolyzed structure 0.3%
CGE isomer 0.1%
CGECH + Epi adduct < 0.1%

Reliability : (2) valid with restrictions
Flag : confidential, Critical study for SIDS endpoint
20.11.2006 (14)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Ames test
System of testing : Salmonella typhimurium TA98, TA100, TA 1535, TA 1537, TA 1538
Test concentration : 2, 8, 40, 200, 1000 ug/plate
Cycotoxic concentr. : TA 1538 and TA100 showed complete inhibition at 5000 ug/plate; TA 1538 showed moderate toxicity at 1000 ug/plate
Metabolic activation : with and without
Result : negative
Method : OECD Guide-line 471
Year :
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Result : The results of MA-61 was negative in all S. typhimurium strains tested in activation and non-activation assays.
Test condition : MA-61 was dissolved in DMSO. There was 0.7ml of S-9 supernatant per 1.0ml of S-9 mix used in the rat liver microsomal activation system.
Reliability : (1) valid without restriction
Flag : confidential, Critical study for SIDS endpoint
20.11.2006 (15)

Type : Ames test
System of testing : Salmonella typhimurium
Test concentration : 10, 100, 1000 ug/plate
Cycotoxic concentr. : 1000 ug/plate
Metabolic activation : with and without
Result : positive
Method : other: similar to OECD Guide-line 471
Year :
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Result : The test substance was positive in the Salmonella typhimurium reverse mutation assay (Ames test) in strains TA 1535 and TA 100 in the absence, and in strain TA 1535 in the presence of rat liver metabolic enzymes. However the mutagenic activity was significantly reduced with the addition of metabolic activation from either of the liver homogenates.
Test condition : S-9 liver homogenates were prepared from the livers of phenobarbital induces and Aroclor-induced rats.
Reliability : (2) valid with restrictions
Flag : confidential, Critical study for SIDS endpoint
20.11.2006 (16)

Type	:	DNA damage and repair assay
System of testing	:	human lymphocytes
Test concentration	:	10, 100, 1000 ppm
Cycotoxic concentr.	:	1000 ppm
Metabolic activation	:	without
Result	:	positive
Method	:	other: similar to EPA OPPTS 870.5550
Year	:	
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4

Method : The test substance was dissolved in DMSO. Positive control was 4-nitroquinoline-1-oxide.
The test substance was added to cultures containing human mononucleated cells in G-0 phase and incubated for 5 hours at 37 degrees C. Cell viability was determined by trypsin blue exclusion. DNA synthesis was determined by measuring tritiated thymidine incorporation by liquid scintillation spectroscopy. Specimens were stained with Giemsa stain and grain counts were performed over 400 nuclei per concentration.
Statistical analysis consisted of Duncan's Least Significant Difference Test (LSD) performed at the 0.5 level.

Result : In an unscheduled DNA-repair assay in isolated human lymphocytes, the test substance induced a significant increase in unscheduled DNA-synthesis at 10 and 100 ppm, but caused a significant reduction at 1000 ppm due to its cytotoxic effects.

Reliability : (2) valid with restrictions
Flag : confidential, Critical study for SIDS endpoint
20.11.2006 (16) (17)

Type	:	Ames test
System of testing	:	Salmonella TA98, TA100, TA 1535, and Saccharomyces
Test concentration	:	0.001 to 5 ul/plate
Cycotoxic concentr.	:	5 ul/plate
Metabolic activation	:	with and without
Result	:	positive
Method	:	other: similar to OECD Guide-line 471
Year	:	
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4

Result : The test substance exhibited genetic activity with TA1535 in activation and non-activation assays and with TA100 in the non-activation assay.

Reliability : (1) valid without restriction
Flag : confidential, Critical study for SIDS endpoint
15.11.2006 (18)

5.6 GENETIC TOXICITY 'IN VIVO'

Type	:	other: Transgenic Mouse Model for in-vivo Mutagenicity Testing
Species	:	rat
Sex	:	male
Strain	:	
Route of admin.	:	dermal
Exposure period	:	5 days
Doses	:	500 mg/kg bw
Result	:	negative
Method	:	other: See Method
Year	:	
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4

Method	:	In the main experiment only one dose level of 500 mg/kg body weight was analysed (including a control group); in this group 5 transgenic male mice were treated on five consecutive days. Animals were sacrificed on day 12 and 33, allowing an expression time of 7 and 28 days following the last application. The expression time is the time required to fully stabilize the induced mutations in the LacZ gene system, and is important for the sensitivity of the assay system. A group of 5 male mice treated dermally for 5 consecutive days with 0.25 mg/kg benzo[a]pyrene served as positive control; these animals were sacrificed on day 12 after the first application. After necropsy the bone-marrow, liver and skin were dissected, flash-frozen and stored in liquid nitrogen until DNA extraction. The isolated DNA was cut with appropriate restriction enzymes, introduced into bacteriophage lambda, and competent Escherichia coli bacteria were transfected with the phages, and plated on soft agar in the presence of phenylgalactose. Plates were scored for clear plaques. Mutation frequencies (MF) were calculated based on scoring in excess of 200,000 plaques per tissue as mean MF per 106 plaques.
Remark	:	This assay is superior to other in-vivo assays as it allows evaluation of any organ including site of first contact for mutagenic activity. The stably integrated bacterial genes can easily be extracted and analysed for gene mutations. An extension of the assay would even allow different types of gene mutations such as base-changes, insertions or deletions to be distinguished. These genes can be quantitatively isolated at any time after exposure, and from any tissue of the mice. These genes can then be integrated into plasmid vectors, multiplied in bacteria, and the presence or absence of LacZ gene expression can be monitored, indicating the presence or absence of gene mutations.
Result	:	All the treated tissue samples were within the control background MF. The positive control showed a MF about 7 times higher than the control, indicating the sensitivity of the test system. The statistically significant increase of the mutation frequency in bone-marrow after the short expression time (but not the long one) was considered not to be biologically significant. In addition, there was no significant difference between the two expression times. From the data it can be concluded that the test substance did not induce mutations in the LacZ gene system introduced stably into the mouse genome, neither at the site of first contact (skin), nor in the liver (major metabolic organ) and the bone-marrow. The study using transgenic Muta Mice with the test substance showed no detectable mutagenic potential in-vivo.
Test condition	:	The transgenic Muta Mice used in this assay contain the bacterial LacZ gene complex stably integrated into each cell of the animals by gene transfer technology.
Conclusion	:	The study using transgenic Muta Mice treated with the test substance showed no detectable mutagenic potential in-vivo. The attendees of the Classification and Labelling Meeting in Arona (Italy) on October 4, 2006 agreed that the Transgenic Rodent Assays are acceptable for classification & labelling and 'The classification & labelling concerning the mutagenicity category 3 is removed for o-CGE'.
Reliability	:	(2) valid with restrictions
Flag	:	confidential, Critical study for SIDS endpoint
20.11.2006		(19)

Type	:	Micronucleus assay
Species	:	mouse
Sex	:	female
Strain	:	B6C3F1
Route of admin.	:	gavage
Exposure period	:	5 days
Doses	:	125mg/kg bw

Result	:	negative
Method	:	other: similar to OECD Guide-line 474
Year	:	
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	Ten female mice were dosed orally with the test substance daily for 5 days. Triethylmelanine, used as a positive control, was injected in 10 mice at a dose of 0.05mg/kg. All animals were sacrificed 4 hours after last treatment. The bone marrow was extracted, smears prepared and stained and analysed according to Schmid (1975). Dunnett's "t" test was used for statistical analysis.
Result	:	The test substance exhibited no significant difference ($p>0.1$) in the % micronuclei from the control. In fact, the % micronuclei from the treated animals was slightly lower than the control animals.
Reliability	:	(2) valid with restrictions
Flag	:	confidential, Critical study for SIDS endpoint

20.11.2006 (16) (20)

Type	:	Dominant lethal assay
Species	:	mouse
Sex	:	male
Strain	:	
Route of admin.	:	dermal
Exposure period	:	3 times per week for 8 weeks
Doses	:	1.5 g/kg
Result	:	positive
Method	:	other: similar to EPA OPPTS 870.5450
Year	:	
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	In a dominant-lethal assay the test substance was administered dermally to B6D2F1 male mice at doses of 1500 mg/kg body weight, three times per week for 8 consecutive weeks. Positive control males were injected once with 0.2ml/kg triethylenemelanine. Following treatment each male was housed with 3 untreated virgin females for two separate breeding periods. All females were sacrificed 13-14 days from the midweek of breeding period without being checked for cervical plugs. At autopsy, females were scored for pregnancy, total number of implants and fetal deaths.
Result	:	A significant decrease in the mean number of implants was recorded suggesting pre-implantation loss; however, there was no increase in post-implantation loss.
Reliability	:	(2) valid with restrictions
Flag	:	confidential, Critical study for SIDS endpoint

20.11.2006 (16) (17)

Type	:	other: body fluid analysis
Species	:	other: see remark
Sex	:	
Strain	:	
Route of admin.	:	gavage
Exposure period	:	4 days
Doses	:	125 mg/kg
Result	:	negative
Method	:	other: see Method
Year	:	
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	In a in-vivo/in-vitro mutagenicity assay, female B6D2F1 and ICR mice were dosed by gavage with 125 mg/kg body weight of test substance in corn oil

for four consecutive days. Urine was collected shortly thereafter and Salmonella typhimurium strain TA 1535 (with and without supplementation of beta-glucuronidase) was exposed to urine samples.
Results were expressed as number of bacterial colonies (revertants to histidine prototrophy) on the treated plates to the number of spontaneous revertants on the control plates.

Result : No mutagenic activity was detected in the urine of B6D2F1 mice neither in the presence or absence of beta-glucuronidase, but urine of ICR mice caused a non-significant increase in mutations in the presence of beta-glucuronidase.

Reliability : (4) not assignable
Flag : confidential, Critical study for SIDS endpoint
20.11.2006 (16) (17)

5.7 CARCINOGENICITY

5.8.1 TOXICITY TO FERTILITY

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

Type : other: Percutaneous penetration
In vitro/in vivo : In vitro
Species :
Sex :
Strain :
Route of admin. : dermal
Exposure period : 24 hours
Frequency of treatm. : single
Duration of test : 24 hours
Doses : 5.0 umol/cm2
Control group :

Method : Percutaneous penetration and metabolism of o-CGE was studied in-vitro in fresh preparations of skin of mouse, rat and human. o-CGE was applied to skin preparations at a concentration of 5.0 umol/cm2 as 14C-labeled product, and percutaneous penetration was measured over a 24-hour period.

Result : Percutaneous penetration showed a plateau was reached at about 15-20 hours with all three skin types. Penetration was highest with mouse skin followed by rat and finally by human skin, whereby about 22% of the applied dose penetrated mouse skin, and 10 % penetrated human skin. Over time the hydrolysis of o-CGE during skin penetration was inhibited, and increasing amounts of unchanged product passed the skin with increasing time.

Reliability : (2) valid with restrictions
20.11.2006 (21)

Type : other: metabolism
In vitro/in vivo :
Species :
Sex :
Strain :
Route of admin. :

Exposure period	:	
Frequency of treatm.	:	
Duration of test	:	
Doses	:	
Control group	:	
Method	:	
Year	:	
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	The metabolic behaviour of 14C-labeled o-CGE was studied using lung and liver cytosolic and microsomal fractions from mouse, rat and human. Both hydrolysis by epoxide hydrolases and conjugation with glutathione by glutathione-S-transferase (GST) were investigated.
Result	:	o-CGE was both a good substrate for GST as well as for epoxide hydrolases. Conjugation with glutathione was more efficient in liver as compared to lung, and highest in mouse liver cytosol. Human lung was more efficient than rat and mouse lung with respect to conjugation. Hydrolysis by epoxide hydrolases was most efficient by human microsomal enzymes, followed by rat and mouse microsomal enzymes. Cytosolic activity was less pronounced, but human cytosolic fractions were more efficient than those of rats and mice. Lung cytosolic fractions were the least efficient, but again human sub-cellular fractions were the most efficient.
Reliability	:	(4) not assignable

20.11.2006 (22)

5.9 SPECIFIC INVESTIGATIONS

5.10 EXPOSURE EXPERIENCE

5.11 ADDITIONAL REMARKS

6.1 ANALYTICAL METHODS

6.2 DETECTION AND IDENTIFICATION

7.1 FUNCTION

7.2 EFFECTS ON ORGANISMS TO BE CONTROLLED

7.3 ORGANISMS TO BE PROTECTED

7.4 USER

7.5 RESISTANCE

8.1 METHODS HANDLING AND STORING

8.2 FIRE GUIDANCE

8.3 EMERGENCY MEASURES

8.4 POSSIB. OF RENDERING SUBST. HARMLESS

8.5 WASTE MANAGEMENT

8.6 SIDE-EFFECTS DETECTION

8.7 SUBSTANCE REGISTERED AS DANGEROUS FOR GROUND WATER

8.8 REACTIVITY TOWARDS CONTAINER MATERIAL

(1) Product data sheet

(2) Ciba-Geigy Ltd. Project No. 894007. 1989. Report on the test for ready biodegradability of TK10410 in the modified Sturm test. [CD:6/144/42]

(3) Ciba-Geigy Ltd. Project No. 894010. 1989. Test for the acute toxicity of kresylglycidylether = DY 023 (TK 10410) to Zebra-fish (Brachydanio rerio). [CD:5/115/9]

(4) Ciba-Geigy Ltd. Project No. 894008. 1989. Test for the acute toxicity of kresylglycidylether = DY 023 (TK 10410) to Daphnia magna.[CD:5/115/7]

(5) Ciba-Geigy Ltd. Project No. 894009. 1989. Report on the test for inhibitory concentration of TK 10410 on aerobic bacteria. [CD:5/115/10]

(6) Ciba-Geigy Ltd. Project No. Siss 1179. 1972. Acute oral LD 50 of TK 10410 in the rat. [CD:5/97/160]

(7) Ciba-Geigy Ltd. Project No. Siss 6432. 1978. Acute inhalation toxicity in the rat of TK 10410 aerosol. [CD:5/97/164]

(8) Ciba-Geigy Ltd. Project No. Siss 1179. 1972. Acute dermal LD 50 of TK 10410 in the rat. [CD:5/97/161]

(9) Ciba-Geigy Ltd. Project No. Siss 4796. 1975. Skin irritation in the rabbit after single application of TK 10410. [CD:5/97/158]

(10) Ciba-Geigy Ltd. Project No. Siss 1179. 1972. Irritation of TK 10410 to the rabbit eye. [CD:5/97/159]

(11) Ciba-Geigy Ltd. Project No. 894320. 1989. Skin sensitization test in the Guinea Pig: Maximisation Test TK10410 (Araldit DY 023). [CD:6/144/46]

(12) Industrial Bio-test Laboratories. No. 8537-08293. 1976. Human Repeated Insult Patch test with liquid sample TK 10410. [CD 4/85/25]

(13) Ciba-Geigy Ltd. Project No. Siss 4796. 1975. Skin sensitizing (contact allergenic) effect in guinea pigs of TK 10410. [CD:5/97/172]

(14) Ciba-Geigy Ltd. Project No. Siss 6432. 1978. 21-day aerosol inhalation study in rats with TK10410. [CD:6/144/47]

(15) Pharmakon Laborartories . 1978. Ames Salmonella/microsome plate test (with and without activation) on Ciba-Geigy MA-61 (Araldite DY023). [CD 5/97/167]

(16) Legator, Marvin. 1977. Integrated mutagenicity testing Program. The University of Texas Medical Branch, Galveston Texas, USA [CD 5/97/168]

(17) Pullin, T.G. 1977. Integrated Mutagenicity Testing Program on Several Epoxy Compounds. DOW Chemicals Company (unpublished results).

(18) Litton Bionetics. Project No. 20838. 1978. Mutagenicity evaluation of MA-61 (Araldite DY023) in the Ames Salmonella/microsome plate test. [CD 5/97/170]

(19) Ballantyne, M. 2000. O-Cresyl glycidyl ether: induction of LacZ- mutations in tissues of treated Muta Mice. Covance Laboratories Ltd., Harrogate, England. (unpublished report).

(20) Schmid, W. 1975. The Micronucleus Test. Mut. Res. 31:9-15.

(21) Boogaard, P.J. et al. 2000. Dermal penetration and metabolism of five glycidyl ethers in human, rat and mouse skin. Xenobiotica 30 (5), 469-483

(22) Boogaard, P.J. et al. 2000. Metabolic inactivation of five glycidyl ethers in lung and liver of humans, rats and mice in vitro. Xenobiotica 30 (5), 485-502.

10.1 END POINT SUMMARY

10.2 HAZARD SUMMARY

10.3 RISK ASSESSMENT

HUNTSMAN

Advanced Materials

DATA SHEET

Araldite® DY-K

Reactive Diluent for Epoxy Resins

General

Liquid epoxy resins, based on bisphenol A and epichlorohydrin, are sometimes too viscous for use in certain applications. The viscosity of liquid resins can be reduced by the addition of solvents, plasticizers or reactive diluents. When formulating thick coatings, the incorporation of solvents is not recommended, since it is impossible to drive them off completely. Plasticizers cannot be tolerated in many electrical applications or where heat resistance properties are required.

Reactive diluents are free-flowing compounds of low molecular weight (130-210) that take part in the curing reaction and considerably decrease the viscosity of an epoxy resin system. Although the addition of reactive diluents will always have some influence on the end properties of the cured resin/hardener system, they represent the best choice of viscosity reduction.

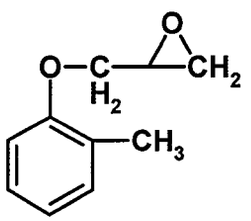
Araldite DY-K is less volatile and shows good resistance to water.

Araldite DY-K can be added to epoxy resins in quantities of 2-20% by weight. Additions of more than 20% cause considerable deterioration the properties of the cured system.

Chemical Description

Araldite DY-K is cresyl glycidyl ether (CGE).

Chemical Structure



Applications

Coatings	Castings
Floorings	Impregnating
Adhesives	Tooling

Advantages

Reducing viscosity of epoxy systems by addition of reactive diluents will afford the following advantages:
Easier handling and mixing
Higher filler loading
Improved wetting characteristics

Typical Properties*

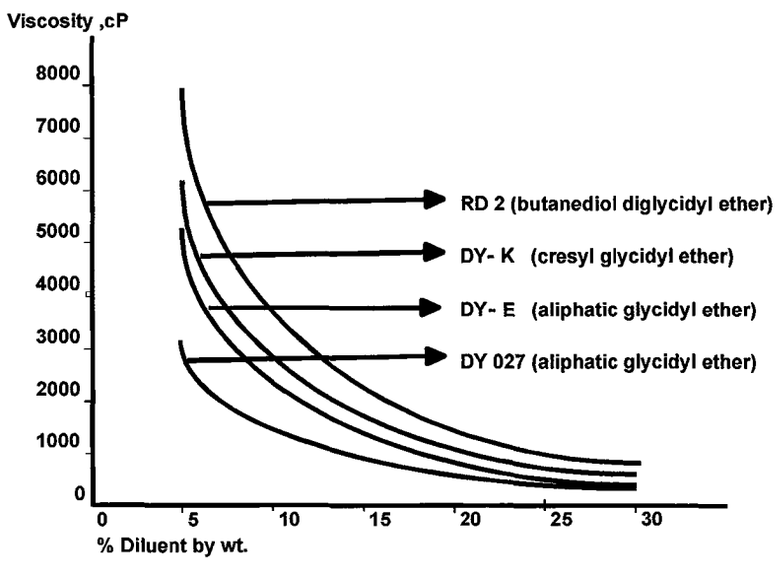
Visual Appearance	Clear, no contamination
Color, Gardner, max	1
Epoxy Value, eq./kg	5.2 - 5.7
Epoxy Equivalent, g/eq.	175 - 190
Viscosity @ 25°C (77°F), mPa s (cPs)	5 - 25
Density @ 25°C (77°F), g/cm^3 (lb/gal.)	1.08 (9.0)
Flash Point, Closed Cup, °C (°F)	116 (240)

* Typical properties are based on Huntsman's test methods. Copies are available upon request.

Formulations

The graph below shows the effect of various Huntsman reactive diluents used with Araldite GY 6010.

Viscosity Reduction Curves



Mixing Procedures

All reactive diluents should be mixed with the resin first. This mixture can be stored for several months. Fillers and hardeners are incorporated just before processing, the latter being stirred in last. Pot life and curing conditions depend on the choice of the epoxy resin and hardener. Since the reactive diluent also has to be cured, it is necessary to adjust the amount of the hardener which is calculated as follows:

The mixing ratio of Araldite GY 6010 and Aradur® 956-2 is 25 phr, i.e., it takes 25 parts by weight of the Aradur 956-2 to cure 100 parts by weight of Araldite GY 6010. When added a reactive diluent, e.g., Araldite DY-K, the hardener ratio should be varied in the same proportion as the epoxy value of the resin / diluent mixture.

Packaging and Storage

Araldite DY-K is supplied in 440-pound steel drums. This material should be stored at room temperature in the original sealed container (actual expiration date appears on the label).

Handling/Safety Precautions

Warning! Skin and eye irritant. May cause dermatitis and sensitization.

Avoid contact with eyes, skin and clothing.
Avoid breathing vapor, mist or spray.
Promptly remove wet, contaminated, non-impervious clothing; wash before re-use.
Destroy contaminated leather and absorbent shoes.
Use with good ventilation.
Store in cool, ventilated, dry area in closed containers.
Wash thoroughly after handling.

Read Material Safety Data Sheet Before Using.
For Industrial Use Only

First Aid

In case of contact:

Eyes: Immediately flush with water for at least 15 minutes.

Skin: Promptly wash thoroughly with mild soap and water.

Inhalation: Remove to fresh air. If breathing is difficult, give oxygen.

Ingestion: If conscious, give large quantities of water and get medical attention.

Important

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Note

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Araldite® DY-K
June, 2004

CD 6/144/42

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CIBA - GEIGY Ltd.
CH-4002 Basle, Switzerland
Ecotoxicology

Project No.: 89 40 07

REPORT ON THE TEST FOR READY BIODEGRADABILITY OF TK 10410 IN THE MODIFIED STURM TEST

(OECD-GUIDELINE No. 301 B) (Paris 1981) *

Sponsor :	CIBA - GEIGY Ltd., KC - Division represented by Dr. A. von Schulthess
Test substance :	TK 10410
Generic / Trade name :	KRESYLGLYCIDYLETHER = DY 023
Code Number :	TK 10410
Batch Number :	611262
Study director	U. Bader 26/04/89
Reported	26/04/89
Approved by	Dr. A. de Morsier 09/05/89
Archives	CIBA - GEIGY Ltd., Basle, R - 1066.K.247

* The EEC Directive 79/831 Annex V Part C 5.2 was established according to the Guideline referenced

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Project No.: 89 40 07

1. Summary

Study :	Determination of the biodegradability by measurement of the carbon dioxide formation in per cent of $ThCO_2$ (Theoretical Carbon dioxide) calculated from the ThOC (Theoretical organic carbon) or TOC (Total organic carbon).
Test substance :	TK 10410
Test system :	Bacteria collected from a sewage treatment plant.
Duration :	28 days
Temperature :	22 ± 2°C
Exposure :	OECD-Guideline for Testing of Chemicals No.: 301 B (May 1981)
Deviations :	The volume of the test solution was reduced from 3.0 l to 1.5 l. The CO_2 formed by biodegradation was absorbed with NaOH and determined on a carbon analyzer. Due to the poor solubility of the test substance in water, an emulsifier was used to achieve a better distribution in the medium. The test substance was added to the medium, homogenized with Nonylphenol 10E05P0.
Results :	The biodegradation calculated as percentage of measured amount of carbondioxide over the theory was: 10.8 mg test substance/l = 4% in 28 days. 20.47 mg test substance/l = 3% in 28 days.
Conclusion :	TK 10410 is not biodegradable in this test.

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2. Introduction

Aim : At the request of the sponsor a test for ready biodegradability was conducted. This report describes the experimental techniques and the results obtained in this study to determine the biodegradation of TK 10410 by bacteria collected from a sewage treatment plant.

Reference : OECD-Guideline for Testing of Chemicals No.: 301 B (May 1981)

Deviations : The volume of the test solution was reduced from 3.0 l to 1.5 l.
The CO_2 formed by biodegradation was absorbed with NaOH and determined on a carbon analyzer.
Due to the poor solubility of the test substance in water, an emulsifier was used to achieve a better distribution in the medium. The test substance was added to the medium, homogenized with Nonylphenol 10E05P0.

Test begin : 13/03/89 (preaeration)
14/03/89 (addition of test substance)

Test end : 11/04/89

Remarks : The test substance was identified on the basis of the code number.

3. Personnel

Study director : U. Bader

Technical personnel : A. Lier (assistant)
E. Dannullis (assistant)

The job descriptions and the summaries of training and professional experience for all personnel participating in this study are archived in the test facility.

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4. Materials and Methods

Test substance :	TK 10410
Batch No.:	611262
Appearance :	liquid
Purity :	COMM. GRADE
Determined by :	by the sponsor
Solubility :	insoluble in water
Received :	13/01/89
Storage :	room temperature
Stability :	01/91
Reference substance :	Aniline MERCK No.: 1261
Test system :	Bacteria collected from activated sludge of the sewage treatment plant of CH - 4153 Reinach on 13/03/89. The preparation was carried out according to the method described in the guideline.
Exposure	
Vessels :	2 Liter flasks (dark brown glass) equipped with gas inlet and magnetic stirrer.
Water :	The test medium was prepared according to the method described in the guideline
Temperature :	22 ± 2°C
Duration :	28 days
Air :	≈ 25 ml/min free of carbon dioxide.

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Test concentrations

Reference substance :	20 mg/l
Test substance :	10.8 mg test substance/l 20.47 mg test substance/l 1200 ml of the mineral solution with the inoculum were aerated for 24 hours in the test vessel. In 300 ml mineral solution 0.5 ml Nonylphenol 10EO5PO (solution of 30 mg in 100 ml bidist. water) and 16.2 rsp. 30.7 mg of test substance were added and homogenized. This solution was given to the test vessel which was immediately connected to the CO_2 traps. 10 mg test substance are equivalent to 7.32 mg organic carbon. This calculation is based on the chemical formula: $C_{10}H_{12}O_2$
Blank:	Water as specified in the guideline containing 0.5 ml of the Nonylphenol 10EO5PO solution .
Measurements :	Determination of the initial CO_2 of the 0.05 N sodium hydroxide and the CO_2, absorbed in the absorbers filled with 200 ml 0.05 N sodium hydroxide on the days 3, 8, 14, 17, 20, 24, 27 and 28.

5. Calculations

The biodegradation was calculated on the basis of the theoretical carbon content of the test substance and the cumulative quantities of carbon dioxide determined on the days of measurements. For the calculation the formula given in the guideline was used.For the blank values, the blank with the vehicle was used.

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6. Results

6.1. Theoretical carbon dioxide formation

Reference substance : 20 mg/l ≙ 85.061 mg CO_2/1.5 liter.

Test substance : 10.8 mg/l ≙ 43.42 mg CO_2/1.5 liter.
Test substance : 20.47 mg/l ≙ 82.28 mg CO_2/1.5 liter.

6.2. Carbon dioxide : See table 1 page 8

6.3. Biodegradation

Reference substance : The biodegradation was calculated as : 20 mg/l = 84.2% in 28 days.

Test substance : The biodegradation was calculated as : 10.8 mg test substance/l = 4% in 28 days.
20.47 mg test substance/l = 3% in 28 days.

7. Conclusion

According to the definition of the OECD expert group on biodegradation and bioaccumulation given in the OECD-Guideline for Testing of Chemicals (1981) TK 10410 is not biodegradable in this test.

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Table 1

Day	CO2 evol. Blank [mg]	CO2 evol. Blank [mg]*	CO2 evol. Blank+Vehicle [mg]	CO2 evol. Blank+Vehicle [mg]*	Ref.: Aniline CO2 evol. Reference [mg]	Ref.: Aniline CO2 evol. Reference [mg]*	20 mg/l Biodegrad. Reference [%]	20 mg/l Biodegrad. Reference [%]*
3			4.8	4.8		0		0
8			2.2	7.0	45.2	45.2	53.2	53.2
14			1.2	8.1	15.0	60.2	17.6	70.8
17			0.5	8.7	3.0	63.2	3.5	74.3
20			0.4	9.1	1.5	64.7	1.8	76.1
24			0.6	9.7	2.8	67.5	3.3	79.3
27			0.5	10.2	2.1	69.6	2.5	81.8
28			0.8	11.1	2.0	71.6	2.4	84.2

* = cumulative values

For calculation of the degradation the Blank+Vehicle is used.

Test substance : TK 10410

Day	Concentration 11 mg/l CO2 evolution [mg]	CO2 evolution [mg]*	Biodegrad. [%]	Biodegrad. [%]*	Concentration 20 mg/l CO2 evolution [mg]	CO2 evolution [mg]*	Biodegrad. [%]	Biodegrad. [%]*
3	0	0	0.1	0	0.8	0.8	0.9	1
8	0.5	0.5	1.1	1	0.5	1.3	0.7	2
14	0.2	0.7	0.5	2	0.4	1.8	0.5	2
17	0.1	0.8	0.2	2	0.4	2.1	0.4	3
20	0.1	0.9	0.2	2	-0.1	2.1	-0.1	2
24	0	0.8	-0.1	2	0.2	2.2	0.2	3
27	0.1	0.9	0.2	2	0.5	2.7	0.6	3
28	1.0	1.9	2.3	4	-0.1	2.6	-0.1	3

* = cumulative values

The negative degradation values are within the variation of the method.

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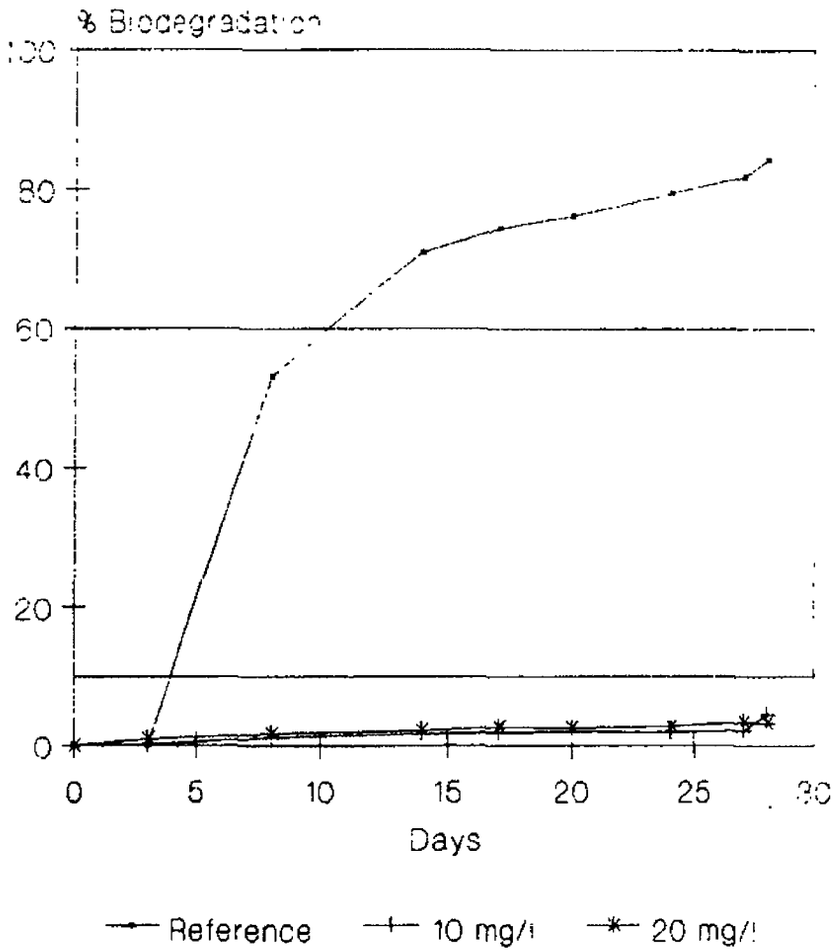
Figure 1 Biodegradation curve of

TK 10410



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Project No.: 894010

CIBA-GEIGY Ltd.
CH-4002 Basle, Switzerland
Ecotoxicology

REPORT

TEST FOR ACUTE TOXICITY

of **KRESYLGLYCIDYLETHER = DY 023 (TK 10410)**

to **Zebra-Fish (Brachydanio rerio)**

OECD-Guideline No. 203, Paris 1984*

Sponsor :	CIBA-GEIGY Ltd., Basle / AD-Division
Represented by :	Dr. A. von Schulthess / R-1002.2.54
	a addite DY 023
Test substance :	KRESYLGLYCIDYLETHER = DY 023 (TK 10410)
Generic/Trade name :	KRESYLGLYCIDYLETHER = DY 023
Code No :	TK 10410
Batch No. :	611262
Reported :	10/04/89
Study director :	..... 10/04/89 ...
	Dr. H. Rufli
Management :	..... 11/04/89
	Dr. A. de Morsier
Archives :	CIBA-GEIGY Ltd., Basle / R-1066.K.247

(* The guideline published in the Official Journal of the European Communities No. L 251 / page 146 , Sept. 16, 1984, was established according to the OECD-Guideline referenced)

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1. SUMMARY

Study :	Determination of LC 50 (96h) ; Concentration at which 50% of the fish population died
Test substance :	KRESYLGLYCIDYLETHER = DY 023 (TK 10410)
Test system :	Zebra-Fish (Brachydanio rerio)
Duration :	96 hours
Temperature :	23 ± 1°C
Exposure :	OECD-Guideline No. 203, Paris 1984 (static procedure)
Deviations :	No stock solution was prepared (see p. 5). Evidence of the test conc. being maintained satisfactorily over the period of the test is not given by analysis.

Results :			
	LC 50 (96 h)	: **13**	**mg/l**
	95% confidence limits	: 11-16	mg/l
	LC 0 (96 h) in test	: 10	mg/l
	LC 100 (96 h) in test	: 18	mg/l

Remarks:

Values are based on nominal concentrations (2 significant digits).

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2. INTRODUCTION

Aim :	At the request of the sponsor an acute toxicity study was conducted. This report describes the experimental techniques and the results obtained in this study determining the acute toxicity on fish of KRESYLGLYCIDYLETHER = DY 023 (TK 10410)
Exposure :	OECD-Guideline No. 203, Paris 1984 (static procedure)
Deviations :	No stock solution was prepared (see p. 5). Evidence of the test conc. being maintained satisfactorily over the period of the test is not given by analysis.
Test begin :	09/03/89
Test begin (exposure) :	13/03/89
Test end (exposure) :	17/03/89
Remark :	The test substance was identified on the basis of the code number. The characterisation and analysis were not part of the study and not within the responsibility of the study director.

3. PERSONNEL

Study Director :	Dr. H. Rufli
Technical Personnel :	S. Flury (technician)

The job descriptions and the summaries of training and professional experience for the personnel participating in this study are archived in the test facility (see p.1).

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4. MATERIALS and METHODS

Test substance :	KRESYLGLYCIDYLETHER = DY 023 (TK 10410)
Code number :	TK 10410
Generic/Trade name :	KRESYLGLYCIDYLETHER = DY 023
Batch No. :	611262
Purity : Determined :	COMM.GRADE by the sponsor
Appearance :	liquid
Solubility in water :	insoluble
Received :	13/01/89
Stability :	01/91
Storage :	room temperature
Stock solution :	No stock solution was prepared. The test substance and 4 mg/l alkylphenol-polyglykol-ether were added directly to the water in the tanks.
Test system :	Zebra-Fish (Brachydanio rerio)
Number of fishes :	10 fishes per concentration and control 10 fishes per aquarium
Length :	24 mm (21-27 mm)
Weight :	0.16 g (0.13-0.23 g)
Loading :	0.11 g/l
Feeding :	None
Adaptation :	24 hours / no food 24 hours prior to exposure
Acclimatisation :	47 days
Treatment :	None
Supplier :	West Aquarium / D-3422 Bad Lauterberg

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Exposure :	Glass aquaria of 20 liters filled with 15 liters (36/22/25 cm)
Water :	Dechlorinated tap water (carbon filter)
Hardness :	184 mg CaCO3/l
Temperature :	23 ± 1°C
Aeration :	none
Light :	Fluorescent light, 16 hours daily
Duration :	96 hours
Measurements :	oxygen, pH, temperature daily
Test concentrations (mg/l) :	5.8, 10, 18, 32, 58
Remarks (nominal) :	Calculated amounts of test substance to produce the desired test concentrations were given into the water and were homogeneously distributed. The fish were then transfered into the aquaria.
Remarks :	The test substance appeared homogeneously distributed in the test vessels.
Controls :	Blank: water Vehicle: 4 mg alkylphenol-polyglykol-ether per liter water in the concentration used for the highest test concentration

5. CALCULATION

The LC-50 values were calculated according to BERKSON, JASA 48 (1953), 569-599.
LC-values were graphically determined on gausso-logarithmic probability paper.

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6. RESULTS

ACUTE TOXICITY of KRESYLGLYCIDYLETHER = DY 023 (TK 10410)

LC 50 (96h) calculated :	**13**	**mg/l**
95% confidence limits :	11-16 mg/l	
LC 50 (72h) :	13	mg/l
95% confidence limits :	11-16 mg/l	
LC 50 (48h) :	13	mg/l
95% confidence limits :	11-16 mg/l	
LC 50 (24h) :	13	mg/l
95% confidence limits :	11-16 mg/l	
LC 50 (96h) graphically determined :	**13**	**mg/l**
LC 0 (96h) in test :	10	mg/l
LC 100 (96h) in test :	18	mg/l

Controls : Mortalities in blank : 0 %
Mortalities in vehicle : 0 %

Remarks:

Values are based on nominal concentrations (2 significant digits).

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7. MEASUREMENTS / OBSERVATIONS

Table 1: Mortalities

Concentration nominal mg/l	Mortalities Number of dead fish 24h	48h	72h	96h
Blank	0	0	0	0
Vehicle	0	0	0	0
5.8	0	0	0	0
10	0	0	0	0
18	10	10	10	10
32	10	10	10	10
58	10	10	10	10

Table 2: Symptoms observed at the different test concentrations

Conc. nominal mg/l	Swimming behaviour				Loss of equilibrium				Respiratory function				Exophtalmus				Pigmentation			
	24	48	72	96	24	48	72	96	24	48	72	96	24	48	72	96	24	48	72	96h
Blank	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vehicle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5.8	1	1	2	2	0	0	1	1	0	0	0	0	0	0	0	0	1	1	1	1
10	2	2	3	3	0	2	2	2	0	0	0	0	0	0	0	0	1	1	1	1
18																				
32																				
58																				

Effect : 0: normal
1: slight
2: moderate
3: severe

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Table 3: Measurements (temperature, pH, oxygen) at the different test concentrations of the test substance (mean values if more than one aquarium per concentration was used)

Conc. nominal mg/l	0h Temp °C	pH	O2 %	24h Temp °C	pH	O2 %	48h Temp °C	pH	O2 %	72h Temp °C	pH	O2 %	96h Temp °C	pH	O2 %
Blank	22	7.9	98	22	8.0	91	22	8.2	93	22	8.2	92	22	8.3	89
Vehicle	22	7.9	99	22	8.0	93	22	8.3	93	22	8.3	91	22	8.3	89
5.8	22	7.9	98	22	8.0	94	22	8.3	93	22	8.3	90	22	8.2	85
10	22	8.0	100	22	8.1	94	22	8.3	92	22	8.3	86	22	8.2	81
18	22	7.9	99	22	8.1	97	22	8.4	99						
32	22	7.9	99	22	8.0	99									
58	22	7.9	100	22	8.1	99									

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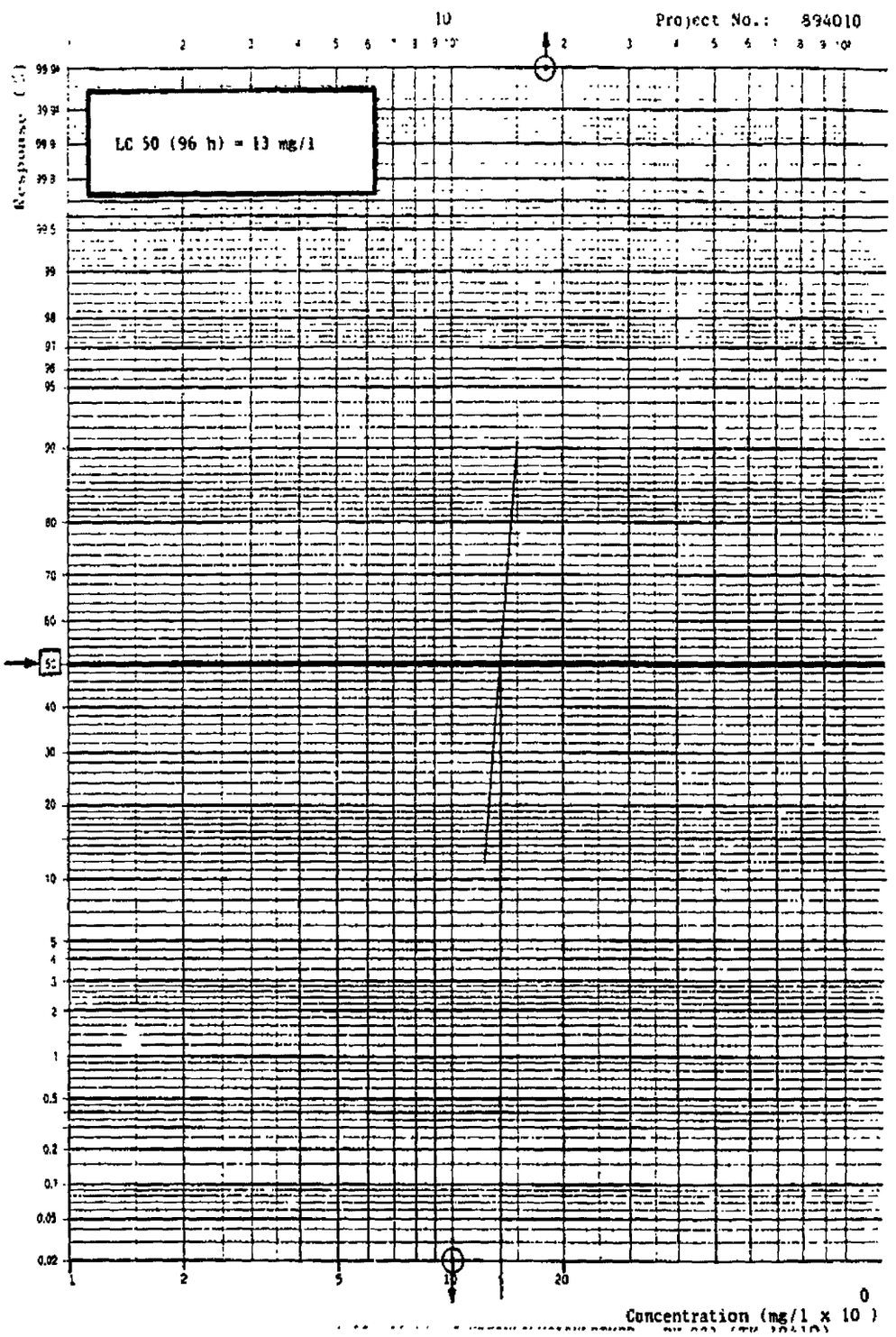

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TEST STRIP

Project No.. 894010

Kresylglycidylether • DY 023 (TK 10410)

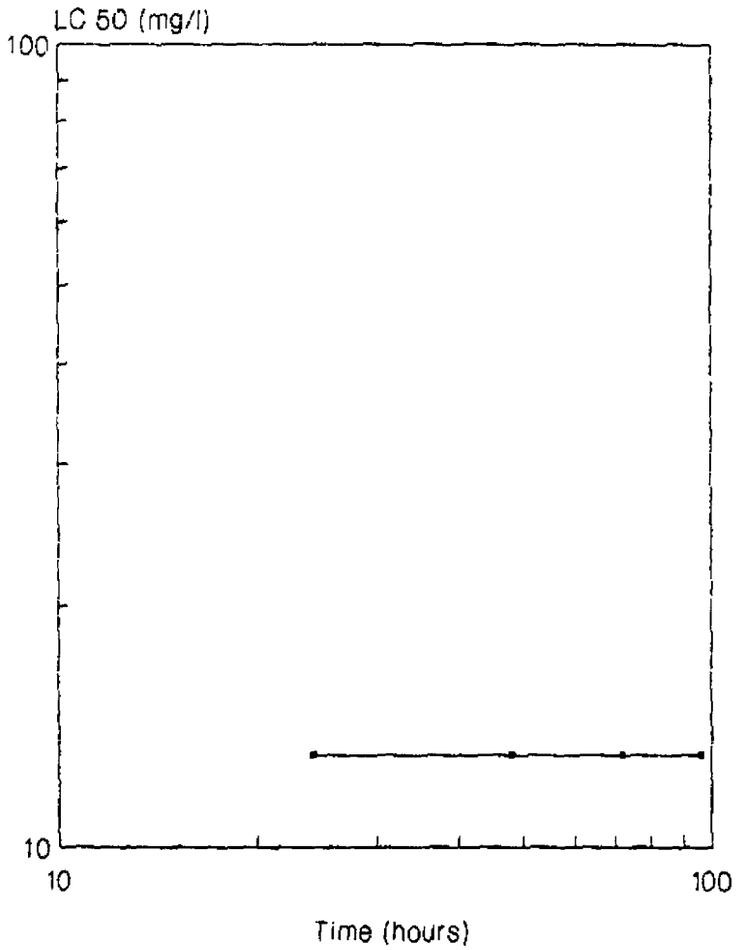

Fig. 2: Time/Toxicity Curve

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Project No.: 894008

CIBA-GEIGY Ltd.
CH-4002 Basle, Switzerland
Ecotoxicology

R E P O R T

TEST FOR ACUTE TOXICITY

of **KRESYLGLYCIDYLETHER = DY 023 (TK 10410)**

to **Daphnia magna**

OECD-Guideline No. 202, Part I, 1984*

Sponsor :	CIBA-GEIGY Ltd., Basle AD-Division
Represented by :	Dr. A. von Schulthess . R-1002.2.54
	Guideline DY 023
Test substance :	KRESYLGLYCIDYLETHER = DY 023 (TK 10410)
Generic Trade name :	KRESYLGLYCIDYLETHER = DY 023
Code No :	TK 10410
Batch No. :	611262
Reported :	22 02 89
Study director :	..... 22/02/89 .....
	Dr. H. Rufli
Management :	..... 23/02/89
	Dr. A. de Morsier
Archives :	CIBA-GEIGY Ltd., Basle . R-1066.K.247

(* The EEC Directive 79 831, Annex V, Part C 5.1.2 was established according to the OECD-Guideline referenced)

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1. SUMMARY

Study :	Determination of EC 50 (24h) : Concentration at which 50% of the daphnia population is immobilized
Test substance :	KRESYLGLYCIDYLETHER = DY 023 (TK 10410)
Test system :	Daphnia magna Straus 1820
Duration :	24 hours
Temperature :	20 ± 1°C
Exposure :	OECD-Guideline No. 202, Part 1, 1984
Deviations :	None

Results :	EC 50 (24 h)	: 16	mg/l
	95% confidence limits	: 14-17 mg l	
	EC 0 (24 h) in test	: 10	mg·l
	EC 100 (24 h) in test	: 32	mg l

Remarks:

Values are based on nominal concentrations (2 significant digits).

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2. INTRODUCTION

Aim :	At the request of the sponsor an acute toxicity study was conducted. This report describes the experimental techniques and the results obtained in this study determining the acute toxicity on Daphnia magna of ARESYLGLICIDYLETHER = DY 023 (TA 10410)
Exposure :	OECD-Guideline No. 202, Part I, 1984
Deviations :	None
Test begin :	16 02 89
Test begin (exposure) :	21 02 89
Test end (exposure) :	22.02 89
Remark :	The test substance was identified on the basis of the code number. The characterisation and analysis were not part of the study and not within the responsibility of the study director.

3. PERSONNEL

Study Director :	Dr. H. Rufli
Technical Personnel :	S. Flury (technician)

The job descriptions and the summaries of training and professional experience for the personnel participating in this study are archived in the test facility (see p.1).

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4. MATERIALS and METHODS

Test substance :	BRESYLGLYCIDYLETHER = DY 023 (TK 10410)
Code number :	TK 10410
Generic Trade name :	BRESYLGLYCIDYLETHER - DY 023
Batch No. :	611262
Purity :	COMM.GRADE
Determined :	by the sponsor
Appearance :	liquid
Solubility (water) :	insol(0.1)
Received :	13 01 89
Stability :	01 91
Storage :	room temperature
Stock solution :	100 mg TK 10410 and 4 mg alkylphenol-polyglykol-ether were mixed with and made up to 1000 ml with water.

Test system

Strain :	Daphnia magna Straus 1820
Source :	CIBA-GEIGY Ltd., Facilities, Basle
Number of daphnia :	20 daphnia per concentration and control 4 replicates of 5 daphnia each
Feeding :	None during the test
Breeding :	Cultures of daphnia are maintained in glass vessels containing approx. 2.5 l of reconstituted water (see below) at 20 ± 1°C (water is renewed partially thrice weekly). At each renewal the daphnia are fed a suspension of green algae (Scenedesmus subspicatus) supplemented by a suspension of TETRAMIN-extract in such quantities that the food is consumed after 24h.
Pretreatment :	24h before the start of the test reproductive daphnia are separated from the young by sieving all individuals through a 800 µm seive. This operation is repeated immediately before the start of the test and the young (0-24h of age) are retained for the test.

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Project No.: 994008

Exposure :	Beakers covered with watch glasses 100 ml solution per beaker
Water :	Reconstituted water produced by dissolving: 65 mg NaHCO3 294 mg CaCl2 ; 2 H2O 123 mg MgSO4 ; 7 H2O 6 mg KCl in 1000 ml bidistilled water. Total hardness : 240 mg CaCO3 l. The water was aerated with clean air for at least 24h before use.
Temperature :	20 ± 1°C
Aeration :	none
Lighting :	Fluorescent light, 16 hours daily approx. 1500 lux
Duration :	24 hours
Measurements :	oxygen, pH, temperature daily (in separate vessels at start of test)
Test concentrations (mg l) :	10, 18, 32, 58, 100
Remarks (nominal) :	Calculated amounts of stock solution to produce the desired test concentrations were given into the water and were homogeneously distributed. The daphnia were then transfered into the beakers.
Remarks :	The test substance appeared homogeneously distributed in the test vessels.
Controls :	Blank: water Vehicle: 4 mg alkylphenol-polyglykol-ether per liter water in the concentration used for the highest test concentration

5. CALCULATION

The EC50-values were calculated according to SPEARMAN-KAERBER in: D.J. Finney, 524-530, London (1964).
EC-values were graphically determined on gausso-logarithmic probability paper

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6. RESULTS

ACUTE TOXICITY of KRESYLGLYCIDYLETHER = DY 023 (TK 10410)

EC 50 (24h) calculated :	**16**	**mg/l**
95% confidence limits :	14-17 mg/l	
EC 50 (24h) graphically determined :	**16**	**mg/l**
EC 0 (24h) in test :	10	mg l
EC 100 (24h) in test :	32	mg l

Controls : Immobilization in blank : 0 %
Immobilization in vehicle : 5 %

Remarks:

Values are based on nominal concentrations (2 significant digits).

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7. MEASUREMENTS OBSERVATIONS

Table 1: Immobilization

Concentration nominal mg.l	Immobilization after 24h in vessel 1	2	3	4	Total number	Total %
Blank	0	0	0	0	0	0
Vehicle	1	0	0	0	1	5
10	0	0	0	0	0	0
18	2	4	4	5	15	75
32	5	5	5	5	20	100
58	5	5	5	5	20	100
100	5	5	5	5	20	100

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Table 7: Measurements (pH, oxygen, temperature)

Concentration nominal mg/l	0h* pH	O2 %	Temp °C	Final measurements** pH	O2 %	Temp °C
Blank	7.6	93	19	7.8	94	21
Vehicle	7.6	93	19	7.8	94	21
10	7.6	93	19	7.8	94	21
18	7.6	93	19	7.8	94	21
32	7.6	93	19	7.8	94	21
58	7.6	94	19	7.8	95	21
100	7.6	94	19	7.8	95	21

* : Measurements in separate vessels (without daphnia)

**: Measurements after the end of exposition; independent of exposure temperature

LEGIBILITY TEST STRIP

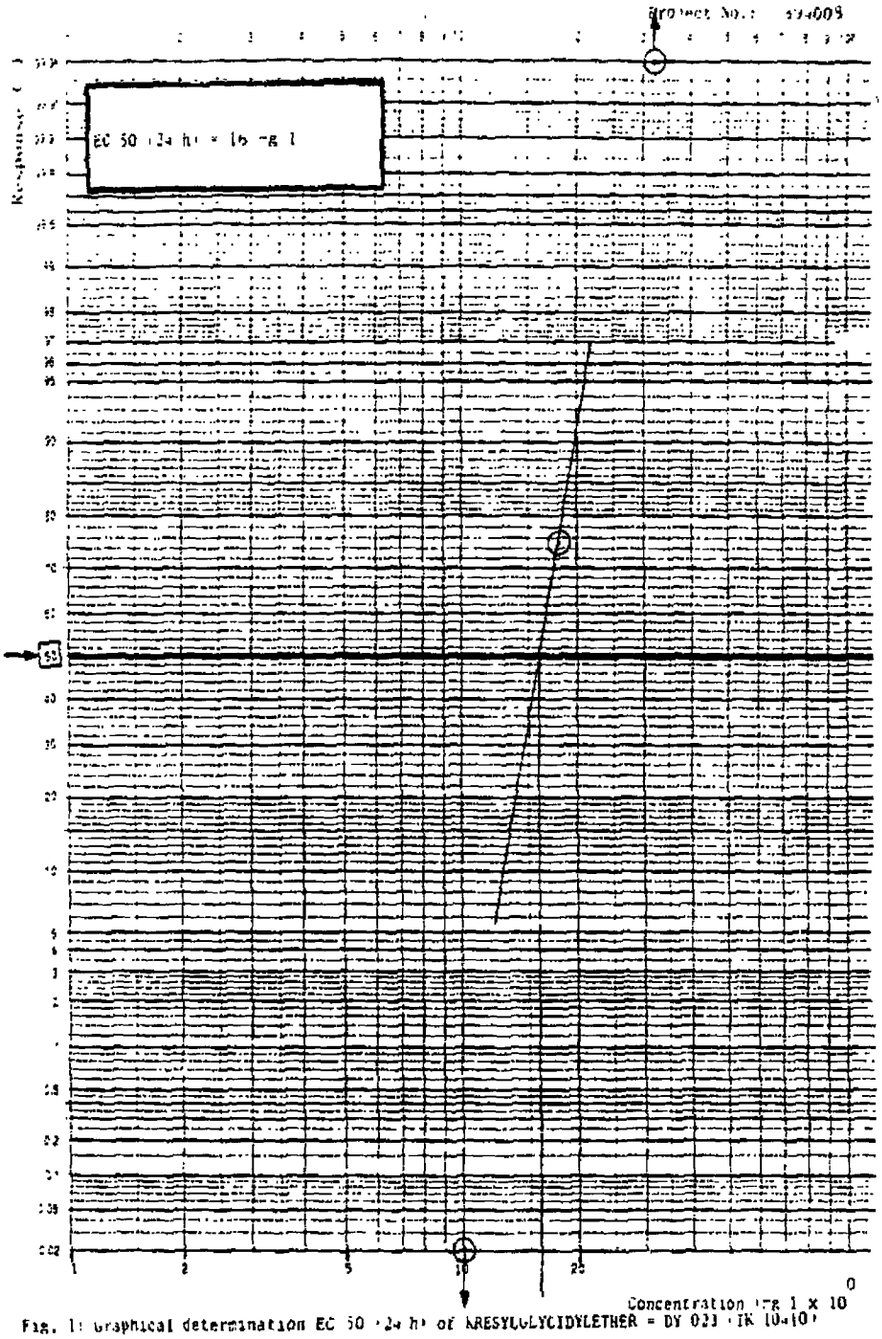

Fig. 1: Graphical determination EC 50 (24 h) of KRESYLGLYCIDYLETHER = DY 023 (TK 10410)

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CD 5/115/10

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CIBA - GEIGY Ltd.
4002 - Basle, Switzerland
Ecotoxicology

Project No.: 89 40 09

REPORT ON THE TEST FOR INHIBITORY CONCENTRATION OF TK 10410 ON AEROBIC BACTERIA

(OECD-GUIDELINE No. 209) (Paris 1984) *

Sponsor :	CIBA - GEIGY Ltd., KU - Division represented by Dr. A. von Schulthess
Test substance :	TK 10410 Araldite DY 023
Generic Trade name :	KRESYLGLYCIDYLETHER = DY 023
Code Number :	TK 10410
Batch No.:	611262
Study director	..... C. Bader
Reported	07/03/89
Approved by	..... Dr. A. de Morsier
Archives	CIBA - GEIGY Ltd., Basle, R - 1066.K.247

* The method published in the Off. J. of the EC, No. L 133, 30.05.1988, p.118 was established according to the OECD Guideline referenced.

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Project No.: 89 40 09

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Project No.: 89 40 09

1. Summary

Study :	Determination of the inhibitory concentration of a chemical substance on the respiration of aerobic waste water bacteria.
Test substance :	TK 10410
Test system :	Activated sludge from a sewage treatment plant.
Duration :	3 hours
Temperature :	20 ± 2°C
Exposure :	OECD Guideline for testing of Chemicals No.: 209 (April 1984)
Deviations :	Instead a centrifuged sludge a settled sludge was used.
Results :	Graphically determined based on nominal concentrations:

IC_{50} > 100 mg/l
IC_{20} > 100 mg/l
IC_{80} > 100 mg/l

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2. Introduction

Aim :	At the request of the sponsor a test for the inhibitory concentration was conducted. This report describes the experimental techniques and the results obtained in this study to determine the inhibitory concentration of TK 10410 on aerobic waste-water bacteria.
Reference :	OECD-Guideline for Testing of Chemicals No.: 209 (April 1984)
Deviations :	Instead a centrifuged sludge a settled sludge was used.
Test date :	21/02/89
Remarks :	The test substance was identified on the basis of the code number.

3. Personnel

Study director :	U. Bader	
Technical personnel :	A. Lier	(assistant)
	E. Dannullis	(assistant)

The job descriptions and the summaries of training and professional experience for all personnel participating in this study are archived in the test facility.

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4. Materials and Methods

Test substance :	TK 10410
Batch No.:	611262
Appearance :	liquid
Purity :	comm. grade
Determined by :	by the sponsor
Solubility :	insoluble in water
Received :	13/01/89
Storage :	room temperature
Stability :	01/91
Reference substance :	3,5-Dichlorophenol
Test system :	Activated sludge collected from the sewage treatment plant of CH - 4153 Reinach on 20/02/89. The preparation was carried out according to the method described in the guideline. The sludge was separated from the aqueous layer by settling instead of centrifugation.

Exposure

Vessels :	250 ml BOD flasks with gas inlet
Water :	Dechlorinated drinking water
Temperature :	20 ± 2°C
Duration :	3 hours
Nutrient solution :	16 g Peptone 11 g Meat extract 3.0 g Urea 0.7 g NaCl 0.4 g $CaCl_2 \cdot 2 H_2O$ 0.2 g $MgSO_4 \cdot 7 H_2O$ 2.8 g K_2HPO_4 per liter dechlorinated drinking water.

JTY
TEST STRIP

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Test concentrations

Reference substance : 32, 10 and 3.2 mg/l

Test substance : 116, 45.5, 23.5, 6.5 and 1.4 mg/l

23.2, 9.1, 4.7, 1.3 rsp. 0.27 mg of the test substance were weighed and added to the test medium. The volume was adjusted to 200 ml with water and aerated for 3 hours.

The sludge concentration in the test bottles was 1.7 g/l.

Measurements : Oxygen consumption per hour in mg/liter with a ORION - Electrode Type 97-08, ORION Microprocessor Ionalizer 901 and plotted on a recorder.

5. Calculations

The inhibitions were calculated on the basis of the measured time depend oxygen consumption of a Blank and Test solution.

6. Results

Graphically determined for TK 10410:

IC_{50} > 100 mg/l
IC_{20} > 100 mg/l
IC_{80} > 100 mg/l

Graphically determined for the reference substance:

IC_{50} = 15 mg/l

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Table 1

Substance	Conc. mg/l	Consumption rate mg/l/h	Inhibition %
Blank 1	0.0	54.86	2
Reference	32.0	14.24	75
Reference	10.0	38.44	31
Reference	3.2	49.07	12
Blank 2	0.0	57.21	-2
TK 10410	116.0	46.75	17
TK 10410	45.5	52.86	6
TK 10410	23.5	50.64	10
TK 10410	6.5	50.12	11
TK 10410	1.4	54.00	4

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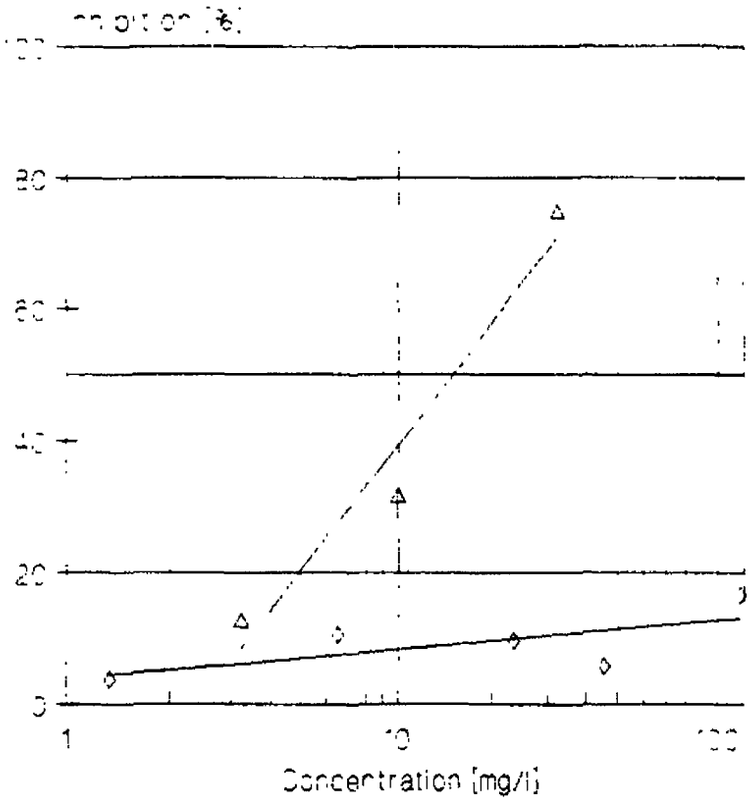
Inhibitory concentration of

TK 10410



LEGIBILITY TEST STRIP

CD 5/97/160

I.D. # 08332
CIBA-GEIGY Limited

March 1st, 1972/ss

PH 2.445 Araldite DY 023

Toxicology Sisseln

Industrial Medicine
DEC 11 1975

ACUTE ORAL LD 50 OF TK 10410 IN THE RAT

Project No.: Siss 1179

Summary and Conclusion

The acute oral LD 50 of TK 10410 in rats of both sexes observed over a period of 7 days is approx. 5800 mg/kg. The compound has therefore a slight acute toxicity to rats by this route of administration.

1. Method

a) Preparation

TK 10410 was weighed into an Erlenmeyer flask on a Mettler balance, it was diluted at 20 and 30% with polyethylene glycol (PEG 400).

b) Animals

The compound was tested on 30 Tif.: RAI/f rats (15 males/15 females), bred under SPF conditions in our own breeding unit. They were 6 to 7 weeks old and weighed 160 to 180 g.

The males and females were segregated and housed in Macrolon cages (type 3) in groups of 5 in a room kept at a constant temperature of 22 ± 1°C and a relative humidity of approximately 55%. They were given water and food (NAFAG, Gossau SG, rat food) ad libitum. The rats were starved during one night before starting the treatment.

p 1/2

2. Results - see table

Dose mg/kg	Concentration % of Formulation	No. of Animals		Deaths within 1 hr		24 hrs		48 hrs		7 days	
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
2150	20	5	5	0	0	0	0	0	0	0	0
3170	30	5	5	0	0	0	0	0	0	0	0
6000	50	5	5	0	0	1	4	2	4	2	4

a) Symptoms

Within 2 to 4 hours of treatment the animals showed dyspnoea, lachrymation, exophtalmus, ruffled fur and curved position.
The animals had recovered within 5 days. They were killed and autopsied after an observation period of 7 days.

b) Autopsy

Dead Animals

Congested liver was seen

Killed Animals

No substance related gross organ changes were seen.

F. Bathe, D.V.M.
Toxicologist

12.6R

CD. 5/97/164

DY. 023

CIBA-GEIGY Limited
Basle, Switzerland

January 11, 1978/wu

Toxicology/Pathology
PH 2.635

I.D. # 12249

Amiblite DY 023

ACUTE INHALATION TOXICITY IN THE RAT OF TK 10410 AEROSOL

Batch No.: 605 497

Project No.: Siss 6432

1. Summary and Conclusion

The LC50 of a 4 hour aerosol exposure for rats of both sexes is 6090 (4795-8512) mg/cubic-meter* air, when evaluated for a 14 day posttreatment observation period. This value according to the classification of Sachsse et al. (1973, 1976)** indicates practically no acute inhalation toxicity for the rat.

2. Method

Inhalation toxicity was tested according to the method of Sachsse et al. (1973, 1976)**.

a) Animals

The test was performed with 40 male and 40 female rats of the Tif: RAIf (SPF) strain, raised on our premises. Before and after the inhalation the animals were kept at a room temperature of 22 ± 1° C, at a relative humidity of 55 ± 5 % and on a 10 hours light cycle day. They received ad libitum rat food - NAFAG, Gossau SG - and water. Prior to treatment they were adapted to our laboratories for a minimum of 4 days and the initial body weight ranged from 190 to 235 grams. The males and females were segregated and kept in Macrolon cages, type 4, (10 animals to a cage).

---

* LC 50 including 95 % confidence limits was calculated by the logit model.

** K. Sachsse, L. Ullmann, G. Voss and R. Hess: Measurement of inhalation toxicity of aerosols in small laboratory animals. In: Proceedings of the Europ. Soc. for the Study of Drug Toxicity. Vol. XV, pp. 239-251, Zurich, June 1973.

K. Sachsse, L. Ullmann, K. Zbinden: Toxikologische Prüfungen von Aerosolen im Tierexperiment: Aus "Chemische Rundschau" 29 (1976), Nr. 38 Seite 1-4.

b) Testing procedures

For inhalation the rats were kept on separate PVC tubes positioned radially around the exposure chamber such that snout and nostrils of the animals only were exposed to the aerosol.

After a 4 hour inhalation the rats were returned to their cages. Physical condition and incidence of death were monitored throughout an observation period of 14 days.

c) Preparation of aerosol

The aerosol was generated by injecting the liquid test material at a rate of 6, 12, 30 and 60 ml/hr into an air stream which was discharged into the exposure chamber through a spray nozzle under a pressure of 2 atm. at a rate of 10 l/min.

The concentration and the particle size distribution of the aerosol in the vicinity of the animals was monitored at 1 hour intervals throughout the aerosol exposure. The concentration was determined gravimetrically by sampling the test atmosphere through a selectron filter of 50 mm diameter and with a pore size of 0.2 µm (Schleicher and Schuell, Feldbach, Switzerland) at an air flow rate of 10 l/min. The size distribution of the particles was measured with a Cascade Impactor with selectron filters of 25 mm diameter and with a pore size of 0.2 µm (Schleicher and Schuell) at an air flow rate of 17.5 l/min. Fig. 1 contains data on the particle size of the test material.

3. Results

a) Rate of deaths - Table 1

b) Signs and symptoms

Within 2 to 4 hours after starting the exposure period the rats in all concentrations showed dyspnoea, exophthalmos, ventral or curved position and ruffled fur. In addition the rats in the highest concentration showed ataxy. The above symptoms were more pronounced in the higher concentrations.

The surviving animals recovered within 4 to 7 days. They were submitted at random to a necropsy whenever they died, survivors at the end of the observation period.

c) Autopsies

Dead Animals: Small isolated hemorrhages in the lungs were seen.

Killed Animals: No substance related gross organ changes were seen.

N. Sachsse D.V.M.

L. Ullmann

Siss 6432

Table 1 - Rate of deaths

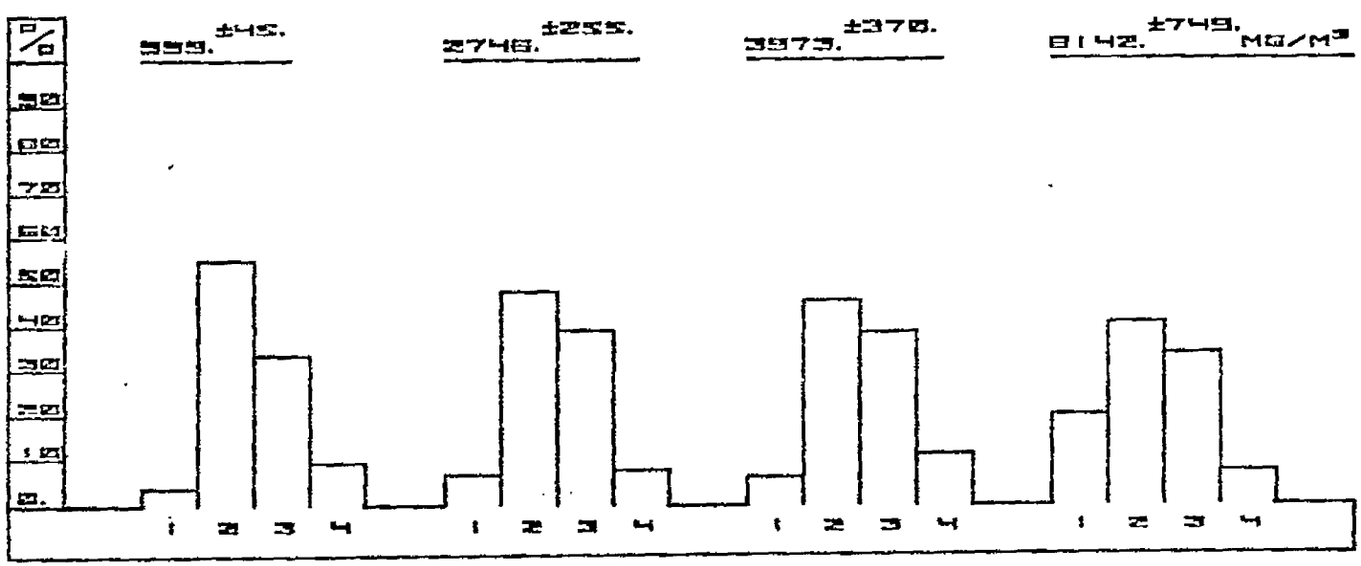
Concentration* mg/m³	Exposure Time (hrs)	No. of Animals		Died within 0-4 hrs		24 hrs.		48 hrs.		7 days		14 days	
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
999 ± 45	4	10	10	0	0	0	0	0	0	0	0	0	0
2746 ± 255	4	10	10	0	0	0	0	0	0	0	0	0	0
3973 ± 370	4	10	10	0	0	1	0	4	0	4	0	4	0
8142 ± 749	4	10	10	6	3	7	4	9	5	9	6	9	6

* Average aerosol concentration, gravimetrically determined.

SIGM.6433. FIG.1.

TK 10410

MEAN PARTICLE SIZE DISTRIBUTION
CASCADE IMPACTOR
AIR FLOW RATE 17.5 L/MIN



1 = ABOVE 7 µM / 2 = 3–7 µM / 3 = 1–3 µM / 4 = 0–1 µM

CO 5/97/161

I.D. # 08335

CIBA-GEIGY Limited Araldite DY023

14.2.72/ss

PH 2.445

Toxicology Sisseln

Industrial Medicine

DEC 11 1975

ACUTE DERMAL LD 50 OF TK 10410 IN THE RAT

Project No.: Siss 11/9

Summary and Conclusion

The acute dermal LD 50 of TK 10410 in rats of both sexes observed over a period of 7 days is >2150 mg/kg.

1. Method

a) Preparation

TK 10410 was weighed into an Erlenmeyer flask on a Mettler balance. It was applied as a concentrate.

b) Animals

The compound was tested on 6 Tif.: RAI/f rats (3 males/3 females), bred under SPF conditions in our own breeding unit. They were 7 to 8 weeks old and weighed 180 to 200 g.

c) Treatment*

Approximately 6 hours before treatment the rats' backs were shaved with an electric razor. The suspension was evenly dispersed on the skin with a Record syringe and covered with aluminium foil, which was held around the trunk with ISO-ELAST plaster. After 24 hours the plaster and the aluminium foil were peeled off carefully and the skin was cleaned with warm water to remove all traces of the suspension.

* Boakes, D.N. and Sanderson, D.M. A method for determining the dermal toxicity of pesticides. Brit. J. Industr. Med., 26, 59-64, 1969

During treatment and for the 7 day observation period the rats were housed singly in Macrolon cages (Type 3) in a room kept at a constant temperature of 22± 1°C and a relative humidity of approximately 50%. They were given water and food (NAFAG, Gossau SG, rat food) ad libitum.

2. Results - see table

Dose mg/kg	Concentration % of Formulation	No. of Animals		Deaths within							
				1 hr		24 hrs		48 hrs		7 days	
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
2150 *	conc.	3	3	0	0	0	0	0	0	0	0

* For technical reasons no higher doses were possible

a) Symptoms

No symptoms and no local skin irritation were observed.

The rats were killed and autopsied after an observation period of 7 days.

b) Killed Animals

No substance related gross organ changes were seen.

R. Lathe D.V.M.
Toxicologist

12.08

CD 5/97/158

I.D. # 08333

CIBA-GEIGY Limited
Basle, Switzerland Araldite DY 023 September 9, 1975/wa

Toxicology/Pathology
PH 2.63

Industrial Medicine

DEC 11 1975

SKIN IRRITATION IN THE RABBIT AFTER SINGLE APPLICATION OF

TK 10410

Batch No.: No. 131583

Project No.: Siss 4796

1. Summary and Conclusion

The primary irritation index, a measure of the acute irritation to the rabbit skin was 5.2. This indicates that the substance is a moderate irritant to the rabbit skin.

2. Method

The procedure used was the patch test technique described in the "Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics" (1959), the US Association of Food and Drug Officials (AFDO).

The test was performed on 3 male and 3 female rabbits of the Russian breed weighing 1.5 to 2 kg. They were housed individually in metal cages, were kept at a constant room temperature of 18 ± 1° C, at a relative humidity of 55 ± 5 % and on a 14 hours light cycle day. The animals received ad libitum standard rabbit feed - NAFAG, Gossau SG - and water. Prior to treatment they were adapted to our laboratories for a minimum of 8 days.

Two days before treatment the entire back and the flank of the rabbits were shaved with an electric clipper and immediately before treatment the shaven skin on the left side was slightly scarified. For treatment gauze patches of 2.5 x 2.5 cm soaked with the test substance were applied to the prepared abraded and non abraded skin.

The patches were covered with an impermeable material and were fastened to the body of the rabbit with adhesive tape. The dressings were removed after a 24 hour exposure.

The skin irritation was scored immediately and 48 hours after removal of the dressings (24 and 72 hours after initiation of treatment). Skin irritation was scored in accordance with the irritation score from table 1.

The severity of skin irritation was rated as follows: A mean value of less than 2 indicated mild, from 2 to 5 moderate and above 6 severe skin irritation.

Score for skin irritation in rabbits (Table 1)

Erythema and eschar formation

No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Maximum possible erythema score	4

Edema formation

No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4
Maximum possible edema score	4

3. Results - Table 2

The primary irritation index is the mean value of the sum of the scores for erythema and edema from all 6 rabbits 24 and 72 hours after initiation of treatment. The maximum possible score is 8.

The primary skin irritation index in this experiment was established to be 5.2.

K. Sachsse DVM

I. Ullmann

Skin irritation in individual rabbits - Table 2

Animal No. Sex	Skin Reaction	24 hrs after application		72 hrs after application	
		intact skin	scarified skin	intact skin	scarified skin
1 ♂	Erythema	3	3	3	3
	Edema	2	2	2	2
2 ♂	Erythema	2	2	1	2
	Edema	2	2	1	1
3 ♂	Erythema	3	3	3	3
	Edema	2	3	2	3
4 ♀	Erythema	3	3	3	3
	Edema	3	3	2	3
5 ♀	Erythema	4	4	4	4
	Edema	2	2	2	2
6 ♀	Erythema	3	3	3	3
	Edema	3	3	3	3

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CIBA-GEIGY Limited
PH 2.445
Toxicology Sisseln I.D. # 08334

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DEC 11 1975

IRRITATION OF TK-10410 TO THE RABBIT EYE

Project No.: Siss 1179

Summary and Conclusion

The primary-irritation index, which serves as a measure of the acute irritation provoked by the substance, was found to be 0 for the cornea, 0 for the iris and 0.8 for the conjunctivae.
Thus TK-10410 is to be considered as a slight irritant to the eye of rabbits.

Test Procedure

The procedure adopted was the technique described in the "Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics" (1959) of the US Association of Food and Drug Officials (AFDO).

The substance was tested on 6 rabbits (3 males/3 females) of the English Silver strain, which were kept separately in V_2A wire cages and fed on NAFAG, Gossau SG, rabbit food and water ad libitum. Only rabbits showing normal ophthalmic findings were included in the test.

Amounts of 0.1 g. of the test substance were introduced into the conjunctival sac of the left eye with a spatula. After application, the eyelids were held open for a few seconds. The right eye served as a control. The treated eyes of three rabbits were each rinsed with 10 ml. lukewarm water about 30 seconds after treatment.

The reactions were appraised with a slit-lamp after 24 hours and after 2, 3, 4 and 7 days on the basis of the "Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics" (1959) of the AFDO..

Siss 1179

Evaluation of the Eye Reactions

Cornea

A. Opacity-degree of density (area most dense taken for reading)

No opacity	0
Scattered or diffuse area, details of iris clearly visible	1
Easily discernible translucent areas, details of iris slightly obscured	2
Opalescent areas, no details of iris visible, size of pupil barely discernible	3
Opaque, iris invisible	4

B. Area of cornea involved

One quarter (or less) but not zero	1
Greater than one quarter, but less than half	2
Greater than half, but less than three quarters	3
Greater than three quarters, up to whole area	4

A x B x 5 Total maximum = 80

Iris

A. Values

Normal	0
Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris still reacting to light (sluggish reaction is positive)	1
No reaction to light, hemorrhage, gross destruction (any or all of these)	2

A x 5

Conjunctivae

A. Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)

Vessels normal	0
Vessels definitely injected above normal	1
More diffuse, deeper crimson red, individual vessels not easily discernible	2
Diffuse beefy red	3

Siss 1179

B Chemosis

No swelling	0
Any swelling above normal (includes nictitating membrane)	1
Obvious swelling with partial eversion of lids	2
Swelling with lids about half closed	3
Swelling with lids about half closed to completely closed	4

C Discharge

No discharge	0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
Discharge with moistening of the lids and hairs just adjacent to lids	2
Discharge with moistening of the lids and hairs, and considerable area around the eye	3

(A + B + C) x 2 Total maximum = 20

Results

The results of the eye irritation test are summarized in tables 1 and 2. The total maximum is 80 for the cornea, 10 for the iris and 20 for the conjunctivae.

The irritation index was found to be 0 for the cornea, 0 for the iris and 0.8 for the conjunctivae.

Siss 1179

Table 1: Eyes not rinsed

Days after Appl.	Rabbit 1 ♂			Rabbit 2 ♂			Rabbit 3 ♂		
	Cornea	Iris	Conjunct.	Cornea	Iris	Conjunct.	Cornea	Iris	Conjunct.
1	0	0	6	0	0	6	0	0	6
2	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0

Table 2: Eyes rinsed

Days after Appl.	Rabbit 4 ♀			Rabbit 5 ♀			Rabbit 6 ♀		
	Cornea	Iris	Conjunct.	Cornea	Iris	Conjunct.	Cornea	Iris	Conjunct.
1	0	0	6	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0

K. Sachsse D.V.M.
Toxicologist

CD 6/144/46

[redacted] Document UK: 19415

Skin Sensitisation Test in the Guinea Pig

Maximisation Test

Test No. 894320

TK 10410 (ARALDIT DY 023)

Report

Study Director:	Dr. phil.II Th. Maurer
Testing Facility:	Dermatotoxicology CIBA-GEIGY Limited 4002 Basel / Switzerland
Test Guideline:	OECD 406 / 84/449/EEC
Starting date:	August 21, 1989
Completion date:	November 22, 1989
Sponsor:	CIBA-GEIGY Limited Plastics Division 4000 Basel / Switzerland

This report contains: 22 pages

0.1. Proprietary Information

Proprietary information of CIBA-GEIGY Limited.
Not to be disclosed to third parties without previous consent
of CIBA-GEIGY Limited.

0.2. Certification of GLP and Verification of the Report

(Certification of Good Laboratory Practice and Verification of a Complete and Unaltered Copy of the Report by the Sponsor)

To the best of my knowledge and belief, the statement of Compliance with Good Laboratory Practice found on page 4 of this report, and signed by the Study Director is truthful and accurate, and this report as provided by the testing facility is complete and unaltered.

For the Sponsor:

Signature: ..... Date: November 24, 1994

0.3. Statement of Compliance with Good Laboratory Practice

To the best of my knowledge and belief this study has been performed in compliance with "Verfahren und Grundsaetze der Guten Laborpraxis (GLP) in der Schweiz" (Good Laboratory Practice (GLP) in Switzerland, Procedures and Principles, March 1986), issued by the Swiss Federal of the Interior which recognize the OECD Principles of Good Laboratory Practice (Council Decision 81/30, adopted on May 12, 1981, and the OECD Recommendation 83/95 concerning the 'Mutual Recognition of Compliance with Good Laboratory Practice', adopted on July 26, 1983).

The competent Swiss Federal Authorities have signed Memoranda of Understanding concerning the mutual recognition of compliance with Good Laboratory Practice with the following agencies:

- the US Food and Drug Administration (April 29, 1985)
- the US Environmental Protection Agency (June 22, 1988)

Study director: Dr. phil. II Th. Maurer

Signature: ..... Date: November 22, 1989

0.4. Signatures

This report represents the results of the investigations compiled by the undersigned:

Study director: Dr. phil.II Th. Maurer

Signature: ..... Date: November 22, 1989

Approved: Prof. Dr. med. D. Mueller
Facility Management

Signature: ..... Date: November 23, 1989

0.5. Reserved Page for Flagging Statements

QUALITY ASSURANCE STATEMENT

Test Substance:	TK 10410 , OP-WMO 366U Araldit DY 023
STUDY TITLE :	SKIN SENSITIZATION TEST IN THE GUINEA PIG MAXIMIZATION TEST
Test Number :	894320

Study Director: Dr. Th. Maurer

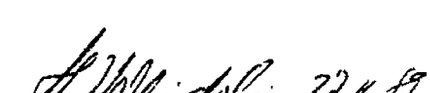
Q.A. Inspector: M. Richert

I hereby certify that the following Quality Assurance activities were performed :

Laboratory Inspections / Final Report Audit

performed	reported to Management
1) 17.08.89	1) 17.08.89
2) 21.08.89	2) 21.08.89
3) 29.08.89	3) 29.08.89
4) 21.11.89 - Final Report Audit	4) 21.11.89

Manager of the Quality Assurance Unit
Dr. H.J. Weideli



(Signature) 22.11.89 (Date)

Skin Sensitisation Test in the Guinea Pig
Test No.: 894320
Test Article: TK 10410

1. SUMMARY AND CONCLUSION

100 % of the animals were sensitised by TK 10410 under the experimental conditions employed.

According to the maximisation grading TK 10410 showed an extreme grade of skin-sensitising (contact allergenic) potential in albino guinea pigs.

2. GENERAL

2.1. Introduction

At the request of the Plastics Division of CIBA-GEIGY Limited, a sensitisation test in albino guinea pigs was performed to determine the contact allergenic potency of TK 10410 in albino guinea pigs.

This test was based on the OECD Guideline No.406, adopted May 12, 1981, by the OECD council, and on Annex V, part B of Council Directive 79/831/EEC (Commission Directive 84/449 EEC of April 25, 1984).

Experimental starting date:	August 21, 1989
Experimental termination date:	September 22, 1989
Testing facility:	CIBA-GEIGY Limited Dermatotoxicology 4002 Basel/Switzerland

2.2. Archives

Protocol and Report	R 1066.434
Paw Data	R 1066.434

2.3. Distribution

Sponsor
Archives
Quality Assurance Unit

2.4. Test Material

Test article:	TK 10410
Batch No.:	OP-WXO 3660
Trade name:	ARALDIT DY 023
Physical properties:	yellowish liquid;insoluble in water
Storage conditions:	room temperature
Validity:	July 1992
Test article received:	July 17, 1989

2.5. Auxiliary Compounds

- Physiological saline (0.9 %), sterile solution (Hausmann, St. Gallen, Switzerland)
- Bacto Adjuvant, Complete, Freund (Difco Lab. Detroit, Michigan USA)
- Vaseline (white petrolatum) Ph. H. VI (Demopharm AG, Biel, Switzerland)
- Sesame oil Ph. H. VI (Siegfried AG, Zofingen)

2.6. Test System

The albino guinea pig is the recommended species for skin sensitisation studies.

Animal strain:	Pirbright White Strain (Tif: DHP)
Breeder:	CIBA-GEIGY Limited Tierfarm 4332 Stein / Switzerland
Date of acclimatisation:	August 14, 1989

2.7. Group Size and Husbandry

The test was performed on 10 male and 10 female guinea pigs per group weighing between 330 to 442 g (approx. 10 weeks old).

The animals were housed individually in Macrolon cages (Type 3), assigned to the different groups by means of random numbers generated by the random number generator, identified by individual ear tags, kept at a constant room temperature of 22±3°C, at a relative humidity of 30 to 70 % and a 12 hours light cycle day.

The animals received ad libitum standard guinea pig pellets - NAFAG No. 845, Gossau SG and fresh water.

All batches of the diet are assayed for nutritive ingredients and contamination level by the manufacturer. Analytical results are available at the animal supply office.

The drinking water is examined periodically by the IWB (Industrielle Werke Basel), results are available to CIBA-GEIGY Limited.

2.8. Sensitivity of strain

The sensitivity of the strain is checked every six month with a known sensitiser, such as 2,4-dinitrochlorobenzene, para-phenylene-diamine or potassium-dichromate.

These tests are not subjected to quality assurance evaluation. The data are archived at R 1066.434.

3. METHODS

3.1. Reason for selection

The maximisation test has been selected, because it is one of the recommended tests in the OECD guideline 406, adopted May 12, 1981, as well as in AnnexV, Part B of Council Directive 79/831/EEC (Commission Directive 84/449/EEC of April 25, 1984 and because the sensitivity of the method is well known. The test has been performed according to the original protocol of Magnusson and Kligman (J. invest. Dermatol. 52, 268-276, 1969, Contact Dermatitis 6, 46-50, 1980).

3.2. Test procedure

3.2.1. Induction Procedure

The induction was a two-stage operation. First, intradermal injections (into the neck region); second, closed patch exposure over the injection sites one week later.

First Induction, intradermal application

Three pairs of intradermal injections (0.1 ml per injection) were made simultaneously into the shaved neck of the guinea pigs as follows:

- adjuvant/saline mixture 1:1 (v/v)

- test article TK 10410 in sesame oil (w/v)

- test article TK 10410 in the adjuvant saline mixture (w/v)

Second Induction, epidermal application

One week later TK 10410 was incorporated in vaseline (w/w) and applied on a filterpaper patch to the neck of the animals (patch 2x4 cm; approx. 0.4 g paste per patch; occluded administration for 48 hours).

3.2.2. Challenge

Two weeks after the epidermal induction application the animals were tested on the flank with TK 10410 in vaseline (w/w) and the vehicle alone (patch 2x2 cm; approx. 0.2 g paste per patch; occluded administration for 24 hours).

3.2.3. Control group

A control group was treated with adjuvant and the vehicle during the induction period. During the challenge period the group was treated with the vehicle as well as with the test article (at least 10 animals) to check the maximum subirritant concentration of the test article in adjuvant treated animals.

3.2.4. Concentrations

Induction

Intradermal application: the concentration for the intradermal injections was selected on account of the solubility of the test article in standard vehicles.
Concentration: 3 %
Vehicle: sesame oil.

Epidermal application: the concentration for the epidermal application was selected on account of the primary irritation potential of TK 10410.
Concentration: 10 %
Vehicle: vaseline (soft white petrolatum).

Challenge

Epidermal application: the concentration for the epidermal application was selected on account of the primary irritation potential of TK 10410.
Concentration: 3 %
Vehicle: vaseline (soft white petrolatum).

The following concentrations have been examined on separate animals for the evaluation of the primary irritant potential and the maximum subirritant concentration of TK 10410:

30, 10 and 3 % of TK 10410 in vaseline.

Erythema reactions were observed with 30 and 10 % TK 10410 in vaseline.

10 % was, therefore, selected for the epidermal induction application and 3 % as maximum subirritant concentration for the epidermal challenge application.

3.3. Observations and records

Induction reactions

After the intradermal and the epidermal induction application irritant reactions are normally induced by the adjuvant, the high test article concentration, or the sodium lauryl sulfate pretreatment. Because most of the reactions are treatment related and not compound related, the reactions are only described in special cases in the section of results.

Challenge reactions

Twenty four hours after removing the dressings, the challenge reactions were graded according to the Draize scoring scale (Appendix 1). A second evaluation was made 48 hours after removing the dressings.

General

The sensitising potential of TK 10410 was classified according to the grading of Magnusson and Kligman (Appendix 2).

The body weight was recorded at start and end of the test.

4. RESULTS

The incidence of positive animals per group and the individual challenge reactions are listed in Tables 1, 2, and 3.

The individual animal weights at start and end of the test are listed in Table 4.

Under the experimental conditions employed, 100 % of the animals of the test group showed skin reactions 24 and 48 hours after removing the dressings.

TK 10410 is, therefore, classified as an extreme sensitiser in albino guinea pigs according to the grading of Magnusson and Kligman.

5. TABLES

TABLE 1

Number of positive animals per group after occlusive epicutaneous application:

Control group:

	after 24 hours	after 48 hours
vehicle control	0 /20	0 /20
test article	0 /10	0 /10

Test group:

	after 24 hours	after 48 hours
vehicle control	0 /20	0 /20
test article	20/20	20/20

TABLE 2

Challenge reactions after epicutaneous administration
(CONTROL GROUP)

DRAIZE Score 24 hours after removal of the dressing

Vehicle control

Male animals	361	362	363	364	365	366	367	368	369	370
Erythema score:	0	0	0	0	0	0	0	0	0	0
Edema score:	0	0	0	0	0	0	0	0	0	0

Female animals	371	372	373	374	375	376	377	378	379	380
Erythema score:	0	0	0	0	0	0	0	0	0	0
Edema score:	0	0	0	0	0	0	0	0	0	0

Test article control

Male animals	361	362	363	364	365	366	367	368	369	370
Erythema score:	0	0	0	0	0	0	0	0	0	0
Edema score:	0	0	0	0	0	0	0	0	0	0

DRAIZE Score 48 hours after removal of the dressing

Vehicle control

Male animals	361	362	363	364	365	366	367	368	369	370
Erythema score:	0	0	0	0	0	0	0	0	0	0
Edema score:	0	0	0	0	0	0	0	0	0	0

Test No.: 894320
Test Article: TK 10410

Female animals	371	372	373	374	375	376	377	378	379	381
Erythema score:	0	0	0	0	0	0	0	0	0	1
Edema score:	0	0	0	0	0	0	0	0	0	1

Test article control

Male animals	361	362	363	364	365	366	367	368	369	371
Erythema score:	0	0	0	0	0	0	0	0	0	1
Edema score:	0	0	0	0	0	0	0	0	0	1

TABLE 3

Challenge reactions after epicutaneous administration
(TEST GROUP)

DRAIZE Score 24 hours after removal of the dressing

Vehicle control

Male animals	401	402	403	404	405	406	407	408	409	410
Erythema score:	0	0	0	0	0	0	0	0	0	0
Edema score:	0	0	0	0	0	0	0	0	0	0

Female animals	411	412	413	414	415	416	417	418	419	420
Erythema score:	0	0	0	0	0	0	0	0	0	0
Edema score:	0	0	0	0	0	0	0	0	0	0

Test article

Male animals	401	402	403	404	405	406	407	408	409	410
Erythema score:	2	2	1	2	3	2	2	2	2	1
Edema score:	1	1	1	1	2	2	1	1	1	0

Female animals	411	412	413	414	415	416	417	418	419	420
Erythema score:	2	2	2	2	2	2	2	2	1	0
Edema score:	1	2	1	2	2	1	1	1	1	0

DRAIZE Score 48 hours after removal of the dressing

Vehicle control

Male animals	401	402	403	404	405	406	407	408	409	410
Erythema score:	0	0	0	0	0	0	0	0	0	0
Edema score:	0	0	0	0	0	0	0	0	0	0

Female animals	411	412	413	414	415	416	417	418	419	420
Erythema score:	0	0	0	0	0	0	0	0	0	0
Edema score:	0	0	0	0	0	0	0	0	0	0

Test article

Male animals	401	402	403	404	405	406	407	408	409	410
Erythema score:	2	1	1	2	2	2	1	2	2	2
Edema score:	1	1	1	1	2	1	1	1	1	1

Female animals	411	412	413	414	415	416	417	418	419	420
Erythema score:	1	2s	2	2	1	1	2s	1	1	2
Edema score:	1	2	1	2	1	1	1	1	2	1

s = scaling

TABLE 4

Individual animal weight in g - males

CONTROL GROUP			TEST GROUP		
Animal No.	weight at start	weight at end	Animal No.	weight at start	weight at end
361	380	658	401	416	[illegible]
362	425	664	402	427	[illegible]
363	389	594	403	431	[illegible]
364	397	640	404	426	[illegible]
365	430	683	405	427	591
366	414	634	406	441	[illegible]
367	405	632	407	426	625
368	426	640	408	434	[illegible]
369	428	667	409	429	699
370	442	757	410	389	581
Mean	413.6	656.9		424.6	[illegible]
Std.Dev.	20.1	42.9		14.9	[illegible]

Individual animal weight in g - females

CONTROL GROUP			TEST GROUP		
Animal No.	weight at start	weight at end	Animal No.	weight at start	weight at end
371	379	570	411	411	614
372	389	645	412	330	[illegible]
373	362	624	413	441	[illegible]
374	383	583	414	404	[illegible]
375	382	594	415	427	[illegible]
376	423	623	416	422	[illegible]
377	391	536	417	386	[illegible]
378	399	556	418	368	[illegible]
379	419	565	419	418	[illegible]
380	380	502	420	382	599
Mean	390.7	579.8		398.9	[illegible]
Std.Dev.	18.6	43.6		33.0	40.6

6. APPENDICES

APPENDIX 1

Evaluation of skin reactions

Evaluation of skin reactions according to Draize in Appraisal of the Safety of chemicals in Foods, Drugs and Cosmetics (1959), The US Association of Food and Drug Officials (AFDO).

Erythema and eschar formation

No erythema.....0
Very slight erythema (barely perceptible).....1
Well defined erythema.....2
Moderate to severe erythema.....3
Severe erythema (beet redness) to slight eschar formation (injuries in depth).....4

Edema formation

No edema.....0
Very slight edema (barely perceptible).....1
Slight edema (edges of area well defined by definite raising).....2
Moderate edema (raised approximately 1 mm).....3
Severe edema (raised more than 1 mm and extending beyond area of exposure).....4

APPENDIX 3

Maximisation grading

Sensitisation rate (%)	Grade	Classification
0 - 8	I	weak
9 - 28	II	mild
29 - 64	III	moderate
65 - 80	IV	strong
81 - 100	V	extreme

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CD 4/85/25

I.D. # 01132 TK10410

Industrial BIO-TEST Laboratories, Inc.
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

REPORT TO

CIBA-GEIGY CORPORATION

HUMAN REPEATED INSULT PATCH TEST WITH FOUR LIQUID SAMPLES

APRIL 13, 1976

IBT NO. 8537-08293

REPORT TO

CIBA-GEIGY CORPORATION

HUMAN REPEATED INSULT PATCH TEST WITH
FOUR LIQUID SAMPLES

APRIL 13, 1976

IBT NO. 8537-08293

I. Introduction

Four liquid samples were received from Ciba-Geigy Corporation for evaluation of their skin irritating and skin sensitizing properties employing 2 panels of 50 human test subjects each. The test materials were identified as follows:

TK 10410 - Clear Liquid
TK 10490 - Clear Liquid
TK 11169 - Clear Liquid
TK 11196 - Clear Liquid

II. Summary

A human repeated patch test utilizing 2 panels of 50 human subjects each (2 materials per panel) was conducted on 4 liquid samples identified as TK 10410, TK 10490, TK 11169 and TK 11196. The composition and disposition of the 2 panels are presented in Tables I and II; scoring criteria are presented in Table III.

Prior to the initiation of patch testing in the 2 panels, the 4 TK materials were screened for the highest nonirritating concentration in 9 volunteer subjects not involved in any other patch tests. Five of the subjects were patched with the 4 TK materials full strength. Patch applications were made using Elastoplast coverlets*; a single patch of each material was placed and left in contact with the skin for 24 hours. The patch sites were graded for irritation upon removal and again after 24, 48 and 72 hours.

Due to severe reactions to the 4 TK materials patched full strength, the remaining 4 subjects were patched at 1% (v/v) in 1% CMC. Reactions noted after patch removal appeared to be very slight to none; consequently, the patch test was initiated in the 2 main panels at 1% (v/v) in 1% CMC.

Twenty-four hours after patch removal, the subjects participating in the screening portion of the study at a 1% concentration exhibited moderate to severe delayed reactions.

A decision was made at this point to terminate patch applications in the main panels and observe all subjects exposed to the 4 TK materials for a period of 72 hours after the first induction patch had been placed.

* Duke Laboratories, South Norwalk, Connecticut

A summary of the results of a single application of TK 10410, TK 11169, TK 10490 and TK 11196 is presented below.

Screening Panel

Product	Conc.	Subject Number	Race Sex Age	Irritation Scores after: Removal Er	Removal Ed	24 hours Er	24 hours Ed	48 hours Er	48 hours Ed	72 hours Er	72 hours Ed
TK 10410	Neat	1	C-M-50	4	1	4	4	4	4	4	4
		2	C-M-31	4	2	4	4	4	4	4	4
		3	C-F-27	4	0	4	4	4	4	4	4
		4	C-F-23	4	4	4	4	4	4	4	4
		5	C-F-24	4	3	4	4	4	4	4	4
TK 10410	1%	1	C-M-30	0	0	0	0	0	0	0	0
		2	C-M-42	0	0	0	0	0	0	0	0
		3	C-F-23	0	0	3	4	4	4	4	4
		4	C-M-31	2	0	2	0	1	0	0	0
TK 11169	Neat	1	C-M-50	0	0	0	0	0	0	0	0
		2	C-M-31	0	0	4	4	4	4	4	4
		3	C-F-27	0	0	0	0	0	0	0	0
		4	C-F-23	4	4	4	4	4	4	4	4
		5	C-F-24	0	0	0	0	0	0	0	0
TK 11169	1%	1	C-M-30	0	0	0	0	0	0	0	0
		2	C-M-42	0	0	0	0	0	0	0	0
		3	C-F-23	0	0	3	3	3	3	3	3
		4	C-M-31	2	1	2	1	2	0	0	0
TK 10490	Neat	1	C-M-50	0	0	0	0	0	0	0	0
		2	C-M-31	0	0	4	4	4	4	4	4
		3	C-F-27	0	0	0	0	0	0	0	0
		4	C-F-23	4	4	4	4	4	4	4	4
		5	C-F-24	0	0	0	0	0	0	0	0
TK 10490	1%	1	C-M-30	0	0	0	0	0	0	0	0
		2	C-M-42	0	0	0	0	0	0	0	0
		3	C-F-23	0	0	4	4	4	4	4	4
		4	C-M-31	2	0	2	0	1	0	0	0

Screening Panel continued

Product	Conc.	Subject Number	Race Sex Age	Irritation Scores after: Removal Er	Removal Ed	24 hours Er	24 hours Ed	48 hours Er	48 hours Ed	72 hours Er	72 hours Ed
TK 11196	Neat	1	C-M-50	4	1	4	4	4	4	4	4
		2	C-M-31	4	0	4	4	4	4	4	4
		3	C-F-27	4	1	4	4	4	4	4	4
		4	C-F-23	4	4	4	4	4	4	4	4
		5	C-F-24	4	2	4	4	4	4	4	4
TK 11196	1%	1	C-M-30	0	0	0	0	0	0	0	0
		2	C-M-42	0	0	0	0	0	0	0	0
		3	C-F-23	0	0	2	2	2	2	2	2
		4	C-M-31	2	0	2	0	1	0	0	0

Er = Erythema
Ed = Edema

C = Caucasian
M = Male
F = Female

Panel I

Summary of Reactions at 1%

Product	Conc.	Subject Number	Race Sex Age	Irritation Score after: Removal Er	Removal Ed	24 hours Er	24 hours Ed	48 hours Er	48 hours Ed	72 hours Er	72 hours Ed
TK 10410	1%	3	C-F-19	1	0	0	0	0	0	0	0
		15	C-M-19	0	0	0	0	0	0	4	4
		17	C-M-29	1	0	0	0	0	0	0	0
		22	C-M-22	0	0	0	0	0	0	4	4
		42	C-F-19	2	0	0	0	0	0	0	0
		44	C-M-36	2	0	0	0	0	0	0	0

Panel II

Summary of Reactions at 1%

Product	Conc.	Subject Number	Race Sex Age	Reading after: Removal Er	Removal Ed	24 hours Er	24 hours Ed	48 hours Er	48 hours Ed	72 hours Er	72 hours Ed
TK 10490	1%	5	C-M-19	0	0	2	0	2	0	2	0
		38	C-F-19	0	0	3	1	3	1	3	1
TK 11196	1%	5	C-M-19	0	0	2	0	2	0	2	0

III. Conclusions

The reactions noted in the 5 subjects initially patch tested with the 4 TK materials full strength were found to persist as active reactions for a period of 4 weeks following the induction application.

One subject (No. 3 - C-F-27) accidentally re-exposed to TK 10410 6 weeks after the first induction patch exhibited a severe reaction at the site of the original patch application.

The reactions noted in the 4 subjects screened at 1% in 1% CMC were found to persist for 3 weeks after the first patch application.

The reactions noted in the 2 main panels were observed after a single application of the respective test materials and were found to be persistent for a period of 2 weeks.

Based on the incidence and severity of the reactions noted, it may be concluded that under the conditions of this study and in the test population utilized, test materials TK 10410, TK10490, TK 11169 and TK 11196 would be potential sensitizing agents in humans.

Respectfully submitted,

INDUSTRIAL BIO-TEST LABORATORIES, INC.

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TABLE I

TEST MATERIALS: TK 10410 and TK 11169

Human Repeated Insult Patch Test

Composition of Test Population

Sex	Age Range (years)	Race	No. of Subjects
Male	18-36	Caucasian	29
Female	18-33	Caucasian	21
		Total -	50

TABLE II

TEST MATERIALS: TK 10490 and TK 11196

Human Repeated Insult Patch Test

Composition of Test Population

Sex	Age Range (years)	Race	No. of Subjects	
Male	18-61	Caucasian	28	
	21	Negro	1	
		Subtotal -		29
Female	18-25	Caucasian	21	
		Subtotal -		21
		Total -		50

TABLE III

TEST MATERIALS: Four Liquid TK Samples

Human Repeated Insult Patch Test

Scoring Criteria for Skin Irritation Reactions

Erythema and Eschar Formation	
No reaction	0
Very slight erythema (barely perceptible)	1
Mild, well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Total possible erythema score	4
Edema Formation	
No reaction	0
Very slight edema (barely perceptible)	1
Slight edema (edge of area well-defined by definite raising)	2
Moderate edema (area raised approximately 1 mm)	3
Severe edema (area raised more than 1 mm and extending beyond area of exposure)	4
Total possible edema score	4

CO: 5/97/172

Basle, Switzerland

DY 023 1/11/78

RESINS

Toxicology/Pathology
PH 2.635
LB. # 12250

TOXICITY
Araldite 04023

SKIN SENSITIZING (CONTACT ALLERGENIC) EFFECT IN GUINEA PIGS OF

TK 10410

Batch No.: 131583

Project No.: Siss 4796

1. Conclusion

Preparation TK 10410 is considered to possess no skin-sensitizing (contact allergenic) potential in albino-guinea pigs.

2. Method

The optimization test[1] was used, an intracutaneous sensitization procedure similar to the method recommended in the "Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics" (1959), the US Association of Food and Drug Officials (AFDO).

a) Animals

The test was performed on groups of 10 male and 10 female guinea pigs of the Pirbright white strain bred on our premises and weighing between 400 to 450 grams. The animals were housed individually in Macrolon cages, type 3, kept at a constant room temperature of 22 ± 1° C, at a relative humidity of 55 ± 5 % and on a 14 hours light cycle day. The animals received ad libitum standard guinea pig pellets - NAFAG, Gossau SG - and water.

---

1 Maurer, Th., Thomann, P., Weirich, E.G. and Hess, R. (1975) The Optimization test in the guinea pig. A method for the predictive evaluation of the contact allergenicity of chemicals.
Agents and Actions Vol. 5 (2), 174-179

b) Testing procedure

During the 1st week volumes of 0.1 ml of the test substance (0.1 %) in saline without adjuvant were injected intradermally on monday at two sites (flank and back), and on wednesday and friday at each one site on the back. Twenty-one hours after administration, the animals were chemically depilated with Butoquick® and three hours later the skin reaction was assessed under artificial lighting (Luxram S 27, "Daylight"). The two largest diameters of the erythematous reaction in vertical alignment were measured (mm) and the skin-fold thickness was determined with a skin-fold gauge (mm). The individual "reaction volume" (ul) was calculated from these values for each reaction from each animal.

During the second and third week of induction the substance TK 10410 was mixed with adjuvant[2] in a 1 : 1 ratio. A total of 6 sensitizing doses of 0.1 ml were injected intracutaneously into the skin of the neck on monday, wednesday and friday. The reactions were not evaluated on these occasions. The test for skin sensitization was performed two weeks after the last sensitizing treatment with the adjuvant mixture. For this test 0.1 ml of the same substance formulation, as employed during the first testing week, was injected intradermally on the previously untreated flank. Twenty-four hours later the reaction site was evaluated in the same manner as during the first testing week.

A group of 20 guinea pigs treated in the same manner with the control vehicle alone served as negative and another group treated with Dinitrochlorobenzene (DNCB) as positive controls.

---

2 Bacto Adjuvant, complete Freund's Adjuvant Difco, Michigan, USA.

For each animal the mean value + one standard deviation was calculated for the reaction volumes of the 4 intracutaneous injections from the first testing week. Skin sensitization was defined to occur of the challenge reaction whenever the reaction volume exceeded the mean value + one SD of the 4 pre-sensitization responses. The number of positive animals in each group was counted with significant differences between treated and control groups assessed by the Fisher test[3].

3. Results

Intradermal injection of the vehicle alone failed to induce skin sensitization.

Table 1 summarizes the incidence of positive reactors for the groups treated with the substance or with DNCB.

Tables 2 and 3 show the reaction volumes for individual animals from each group.

K. Sachsse D.V.M.

L. Ullmann

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3 The exact Fisher test for comparison of the basic probability of two binomial distributions; L. Sachs, Statistische Auswertungsmethoden, Thieme Verlag, Stuttgart, 1971.

Siss 4796

Table 1 Rate of positive reactors for substance and DNCB treated groups and p = level of significant difference with control group.

Compound	Rate of positive reactors	p
DNCB (Dinitrochloro-benzene)	20/20	< 0.001
TK 10410	3/20	> 0.01

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Table 2 - Reaction volumes (µl) after intradermal injection of Dinitrochlorobenzene 0.1 %

Animal No.	INDUCTION Application No. 1	2	3	4	mean ($\bar{x}$)	standard deviation (s)	($\bar{x}$ + s)	after skin sensitization	+ = positive reactor
1 ♂	36	56	29	40	40	11	51	67	+
2	32	39	10	24	27	12	39	405	+
3	44	16	44	6	20	16	36	109	+
4	18	32	6	11	17	11	28	168	+
5	11	8	1	5	6	4	10	315	+
6	54	69	3	28	39	29	68	272	+
7	43	45	12	56	39	19	58	273	+
8	54	12	8	29	26	21	47	185	+
9	11	43	28	28	28	13	41	123	+
10	21	54	6	28	27	20	47	115	+
11 ♀	53	40	25	24	35	14	49	115	+
12	81	32	15	16	36	31	67	148	+
13	19	24	3	3	12	11	23	50	+
14	3	38	24	39	30	10	40	253	+
15	24	13	16	24	19	5	24	202	+
16	4	43	7	20	18	18	36	130	+
17	6	27	10	18	15	9	24	132	+
18	10	38	25	11	24	11	35	97	+
19	4	5	13	5	7	4	11	63	+
20	24	36	10	19	22	11	33	152	+
Group-mean	28	30	13	22				169	

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Table 3 - Reaction volumes (µl) after intradermal injection of TK 10410 0.1 %

Animal No.	INDUCTION Application No. 1	2	3	4	mean ($\bar{x}$)	standard deviation (s)	($\bar{x}$ + s)	after skin sensitization	+ = positive reactor
1 ♂	27	19	65	56	41.8	22.2	64	48	-
2	38	34	0	67	34.8	27.4	62.2	35	-
3	154	63	6	49	68	62.3	130.3	43	-
4	20	92	46	20	44.5	34	78.5	16	-
5	6	80	38	144	67	59.6	126.6	34	-
6	81	15	6	3.6	26.4	36.7	63.1	108	+
7	6.4	22	3.2	86	29.4	38.6	68	65	-
8	50	112	25	50	59.3	37.1	96.4	88	-
9	132	54	4.8	6	49.2	59.8	109	28	-
10	0.4	0	4.8	40	11.3	19.3	30.6	43	+
11 ♀	0.2	1.4	63	45	27.4	31.6	59	6	-
12	113	126	0	43	70.5	59.5	130	10.5	-
13	12.6	34	54	30	32.7	17	49.7	14.4	-
14	4.2	0	99	77	45.1	50.4	95.5	0	-
15	48	8	72	50	44.5	26.7	71.2	34	-
16	69	0.6	24	0	23.4	32.8	55.8	56	+
17	72	70	77	34	63.3	19.7	83	8.4	-
18	0	0.3	18	26	11.1	13	24.1	7.5	-
19	27	0.4	99	38	41.1	41.7	82.8	6	-
20	22	0	42	40	26	19.5	45.5	1.2	-
Group-mean	44.1	36.6	37.3	45.2				32.6	

CD 5/97/107

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I.D. # 10882

December 21, 1978

MA-61 - clear light yellow
liquid - (DY 023 - Ba N 281)
Araldite DY 023

SUMMARY DATA

Study: Ames *Salmonella*/Microsome Plate Test (with and without metabolic activation) on:

Ciba Geigy MA-61

Sponsor: Ciba Geigy Corporation
Saw Mill River Road
Ardsley, New York 10502

Purpose: To evaluate MA-61 for genetic activity in the Ames *Salmonella typhimurium* assay with and without metabolic activation preparation.

Materials and Methods: MA-61 was dissolved in DMSO. Dose levels for the preliminary toxicity screen were 5000, 1000, 200, 40 and 8 µg/plate. Strains TA 1538 and TA 100 showed complete inhibition at 5000 µg/plate. Strain TA 1538 showed moderate inhibition at 1000 µg/plate. Dose levels for MA-61 in the plate assay were 1000, 200, 40, 8, and 2 µg/plate. There were 0.07 ml of S-9 supernatant per 1.0 ml of S-9 mix used in the rat liver microsomal activation system.

Results: The results of MA-61 were negative in strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100 of *Salmonella typhimurium* in the Ames Test.

Submitted by Richard J. Matthews
Richard J. Matthews, Ph.D.
Director of Research

Investigator Robert W. Naismith
Robert W. Naismith, Ph.D.
Study Director

TREATMENT RECORD FILE
INT. MED. MEDICINE

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p. 1/5

PHARMAKON LABORATORIES

MUTAGENESIS SECTION
Ames Test
Salmonella typhimurium

SUMMARY DATA

Client: Ciba Geigy

Material: PA 61

Description: Clear Light Yellow Liquid

Solvent: DMSO

Date Received: December 1, 1978

Date Performed: December 15, 1978
53-54

Pharmakon Reference: 218, pg. 58-60

CONTROLS

Spontaneous Revertants ($\bar{x}$)

	S-9	TA1535	TA1537	TA1538	TA98	TA100
Negative Controls	(-)[1]	13	10	14	14	153
	(+)[2]	7	8	20	18	149
Positive Controls						
N-methyl-N'-nitro-N-nitrosoguanidine	(-)	1201				
9-Aminoacridine	(-)		613			1197
2-Aminofluorene	(+)			730	612	

TEST COMPOUND

Revertant Colonies/Plate ($\bar{x}$)

Dose Level (µg/plate)	S-9	TA1535	TA1537	TA1538	TA98	TA100
1000	-/+	0/0	0/4	2/1	4/2	0/6
200	-/+	26/10	2/7	10/12	7/17	270/176
40	-/+	15/7	5/7	11/18	7/17	179/154
8	-/+	10/6	6/10	8/12	6/18	147/148
2	-/+	4/6	2/10	5/10	5/13	107/147
	-/+					
	-/+					

[1] (-) Without S-9 Aroclor-induced rat liver metabolic activation.

[2] (+) With S-9 " " " " " "

Investigator: Edmund D. Dobb Date December 15, 1978

Read and understood by me this 15 day of December, 1978 Robert W Nesmith
Witness

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EXPERIMENTAL PROCEDURE

Ames Test

Salmonella typhimurium

INTRODUCTION:[1]

The objective of mutagenicity testing is to assess the potential hazard to human beings of compounds or mixtures of compounds due to their interaction with genetical mechanisms with resultant heritable change (mutation). A large body of knowledge in the area of genetics leads to the conclusion that an increase in expressed genetic diseases can occur in the human population as a result of mutagenic chemicals. Mutation is a universal biological mechanism underlying genetic change in all systems. Mutations are generally observed to be deleterious in reference to the normal environment for an organism. The usual target molecule DNA, is chemically the same in all cellular organisms. Therefore, test results from a wide variety of cells and organisms are relevant to the assessment of the mutagenic potential of a chemical for humans. One or a few molecules reacting with germ cell DNA may cause a heritable lesion. There is a variety of genetic mechanisms by which this heritable genetic change can occur. The Ames Test is designed to detect gene mutations, namely, point mutations. These mutations involve changes within a gene which include base pair substitutions, frameshift mutations and small deletions and insertions within the gene. More specifically, the compounds are tested on petri plates with several specially constructed mutants of *Salmonella typhimurium* selected for sensitivity and specificity in being reverted from a histidine requirement back to prototrophy by a wide variety of mutagens. The test has been adapted for use in detecting chemicals which are potential human carcinogens or mutagens by adding homogenates of rat liver directly to the petri plates, thus incorporating an important aspect of mammalian metabolism into the *in vitro* test. In this way, a wide variety of carcinogens requiring metabolic activation can be detected as mutagens.[2]

PURPOSE:

To evaluate test chemicals for genetic activity in the Ames Test using *Salmonella typhimurium* with and without the addition of a mammalian metabolic activation system.

METHODS AND MATERIALS:

Tester Strains

Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98, and TA100 were received from the laboratory of Dr. Bruce N. Ames. These tester strains were maintained in duplicate at -80°C, and serve as a master and stock culture. Fresh cultures for mutagenesis testing are prepared each week by scraping a sterile wooden applicator stick over the surface of the frozen culture, inoculated into nutrient broth and grown for 16 hours in a 37°C shaking bath. Tester strains are routinely checked for the presence of the appropriate genetic markers.

Preliminary Toxicity Screen

A preliminary toxicity screen with two tester strains, one containing the plasmid factor and the other without a plasmid factor, is carried out to determine the test compound's toxicity to the tester strains. The test chemical solubility is determined by suspending in water, ethanol, or DMSO. Minimal glucose plates containing 2 ml of top agar overlay, 0.1 ml of the appropriate tester strain, and 0.1 ml of test chemical are plated at concentrations of 5000, 1000, 200, 40, and 8 µg/plate. Plates are scored for the degree of inhibition of the bacterial lawn as an indication of toxicity.

Induction of Rat liver Enzymes for Carcinogen Activation

Sprague Dawley CD male rats of about 200 grams are given a single i.p. injection of Aroclor 1254. Aroclor 1254, diluted in corn oil, was dosed at 500 mg/kg to each of three rats five days prior to sacrifice. The rats are given drinking water *ad libitum*, and fed Wayne Lab-Blox until 12 hours before sacrifice when the food is removed. The livers are removed aseptically, minced, homogenized, and centrifuged at 9000 g for 10 minutes at 0 - 4°C. The supernatant was decanted, frozen on dry ice and stored at -80°C. Samples are thawed at room temperature prior to use. The amount of S-9 supernatant used for optimum mutagenesis is critical. We routinely check this variable with several compounds to determine the optimum amount for general screening. In general, we find from Aroclor-induced rats that 0.07 ml of S-9 supernatant per ml of S-9 mix is a good amount for general screening.

Mutagenesis Assay

All test chemicals are screened at five dose levels, the highest level showing some degree of toxicity in at least one of the tester strains. These determinations are based on the preliminary toxicity screens. The dose levels are reported in the summary section. Concurrent positive and negative controls are included in each assay. All dose levels and controls are run in duplicate. The routine controls include both direct acting mutagens and mutagens requiring metabolic activation. 0.1 ml of the appropriate bacterial tester strain, 0.1 ml of the sample to be tested and

0.5 ml of the S-9 mix are added to 2 ml of 45° C top agar. The "overlay" is vortexed to assure mixing and poured onto minimal glucose plates. The plates are covered immediately to avoid any light effects on photosensitive chemicals. Within an hour the plates are put in a dark 37° C incubator. After two days the colonies on both test plates and control plates are counted. Slightly larger numbers of spontaneous revertant colonies arise on plates containing the S-9 mix.

RESULTS AND INTERPRETATION:

Reversion colonies for the controls and test substances are found on the summary data sheet. A substance is considered to have a negative response in the test if at least 500 µg or the maximum non-inhibitory level has been tested on the plate and if the number of induced revertants compared to the spontaneous was less than two-fold (i.e. spontaneous 40, revertants would be less than 120).

BIBLIOGRAPHY:

1. Environmental Protection Agency. Mutagenicity Testing Requirements Section of the FIFRA Guidelines. July 12, 1977.

2. Ames, Bruce, N., Joyce McCann and Edith Yamasaki. Methods for Detecting Carcinogens and Mutagens with the Salmonella/Mammalian-Microsome Mutagenicity Test. Mutation Research 31: (1975) 347-364.

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CD 6/144/47

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Document UK: 30434

CIBA - GEIGY LIMITED
BASLE / SWITZERLAND

TOXICOLOGY
GU 2

TK 10'410

21-DAY AEROSOL INHALATION STUDY IN RATS

DECEMBER 5, 1978

CIBA-GEIGY LIMITED
Basle / Switzerland

Toxicology
GU 2

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Toxicology
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Toxicology
GU 2

21-DAY AEROSOL INHALATION STUDY IN RATS WITH TK 10'410

Material: TK 10'410

Subject: 21-day aerosol inhalation study in Rats followed by a 21-day recovery period

Project No.: Siss 6432

Study Director

Dr. med. vet. K. Sachsse

Responsible for the animal experimentation

L. Ullmann

Responsible for the laboratory investigations

B. Luetkemeier B.Sc.

Responsible for the pathology

F. Zak M.D., Ph.D.

Reviewed and approved by

Prof. Dr. med. R. Hess

December 5, 1978/stu

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Toxicology
GT 2

SUMMARY AND ASSESSMENT

Test compound	TK 10'410
Batch No.	605 497
Test species	RAI f SPF rats (RA 25)
No. of animals per experimental group	10 males, 10 females
Route of exposure	inhalation (head exposure)
Concentration levels $\bar{x} \pm SD$	0 mg/m³ (Control) - group 1 53 ± 4 mg/m³ - group 2 152 ± 4 mg/m³ - group 3 305 ± 10 mg/m³ - group 4
Duration of test	21 days
Follow up period	21 days (from control and group 3)
Starting date	11th January 1978
Termination date	22nd February 1978

Reaction to treatment may be summarised as follows:

At 305 + 10 mg/m³

Reduction in food consumption during the first 15 days of the exposure period. Bodyweights were significantly decreased at various points during the exposure period.

Dyspnoe, exophthalmos, lateral or ventral position, bloating of the abdomen and ruffled fur were seen during the entire exposure period.

15 (6 males, 9 females) rats died during the exposure period. In all these animals acute congestion, and occasional haemorrhages in the myocardium, lungs, liver, kidneys, adrenals, pituitary, ovaries and brain were found.

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Toxicology
GU 2

Furthermore, all rats displayed marked congestion of the nasal mucosa with purulent inflammation and ulceration. In 3 of these rats ulceration of the skin of the muzzle was also noted. All animals showed depletion of thymocytes in the thymus, 5 out of the 10 male rats showed reduced spermatogenesis and in 6 animals atrophy of the lymphoid tissue of the spleen was observed.

The organ weights, organ to body weight and organ to brain weight ratios of the animals sacrificed at termination of the treatment period were changed according to the lower body weights of those animals.

At 152 + 4 mg/m³

Reduction in food consumption and body weight gain during the first 8 days resp. the entire exposure period.

Dyspnoe, exophthalmos and ruffled fur were observed during the exposure period.

Less pronounced congestion and purulent inflammation with ulceration of the nasal mucosa were seen in the muzzle in 6 out of 10 rats. The nasal changes proved to be reversible after a recovery period of 21 days. Of this recovery group only one out of 10 rats showed in the muzzle remnants of a partially re-epithelized ulceration, foreign body reaction and chronic inflammatory infiltration at the base. One male rat only showed arrest of spermatogenesis in one testicle. The experimental significance of this finding is doubtful since unilaterally inhibited spermatogenesis is known to occur spontaneously in rats.

At 53 + 4 mg/m³

Reduced food consumption and body weight gain of male rats between days 3 and 10. Dyspnoe, exophthalmos and ruffled fur were observed.

It can be inferred from the observations made during the above study that the "no observable effect level" is below 53 mg/m³ air for male and female rats.

December 5, 1978

Compound: TK 10'410

TEST MATERIAL

Identification:	TK 10'410
Received:	November 25, 1977
Description:	yellow liquid
Batch No.	605497

METHOD

Species	RAI f SPF rats (RA 25)
Husbandry (except for the exposure period)	air conditioned rooms (15 air changes/hours) temperature 22 ± 1°C relative humidity 50 ± 5 % (animal room) groups of 5 animals per cage (typ 3 Macrolon cages)
Food	pelleted standard diet, Nafag No. 890 ad libitum
Water	tap water, ad libitum
No. of animals per group	10 males, 10 females
Initial body weight	166 - 204 g
Age	5 to 6 weeks
Preparation	TK 10'410, used as a concentrate Control: tap water
Frequency of exposure	once/day (6 hours), 15 treatments (except week-ends)
Duration of test	21 days
Follow up period	21 days

Compound: TK 10'410

Route of exposure	Inhalation, according to the method of Sachsse et al. (1973, 1976)*. The animals were kept separately in PVC tubes which were positioned radially around the exposure chambe
Aerosol generation	TK 10'410 was injected with the help of a "Perfusor" (B. Braun, Melsungen/Germany) at rates of 0.33, 0.94 and 1.65 ml/hr. into an air stream discharged into the exposure chambe through a spray nozzle under a pressure of 2 atm. at a rate of 10 l/min. Control: the rats were treated with 1.65 ml/hr of tap water under the same conditions as described above.
Determination of concentration + particle size distribution	In the vicinity of the animals throughout the exposure time.
Concentration	Gravimetrically on selectronfilters, pore size 0.2 µm and 50 mm in diameter (Schleicher and Schuell, Feldbach/Switzerland), airflow 10 l/min., 5 times a day.
Size distribution	Gravimetrically with a 4 stage Cascade Impactor on selectron filters, pore size 0.2 µm and 25 mm diameter (Schleicher and Schuell, Feldbach/Switzerland), airflow 17.5 l/min., twice a day.

Groups and Concentrations

Group	Animals		Animal Numbers		Concentration mg/m³, $\bar{x} \pm$ SD gravimetrically determined
	male	female	male	female	
1	10*	10*	1-10	41-50	0
2	10	10	11-20	51-60	53 ± 4
3	10*	10*	21-30	61-70	152 ± 4
4	10	10	31-40	71-80	305 ± 10

*) 10 (5 males, 5 females) rats of each concentration for a 21-day recovery period.

*) K. Sachsse, L. Ullmann, G. Voss and R. Hess: Measurement of inhalation toxicity of aerosols in small laboratory animals. In: Proceedings of the Europ. Soc. for the Study of Drug Toxicity. Vol. XV, pp. 239-251, Zurich, June 1973.

K. Sachsse, L. Ullmann, K. Zbinden: Toxikologische Prüfungen von Aerosolen im Tierexperiment, "Chemische Rundschau" 29 (1976), Nr. 38, Seite 1

Compound: TK 10'410

Observations and Records

Mortality	daily
Symptoms	daily
Food consumption	twice weekly
Body weight	daily, except weekends
Eye examination	Ophthalmic inspections were performed weekly
Temperature and humidity control in the test chamber	daily

Statistical Analysis

for others than Laboratory Investigations:

For each time point and parameter a uni-variate statistical analysis was conducted. Due to the routine manner of the analysis system parameter free methods were applied. Each treated group was compared to the control group in respect of dispersion and displacement*. In addition a trend test** was applied considering all groups.

for Laboratory Investigations:

Student's "t" test and the analysis of variance were employed to assess the significance of difference between concentration groups and controls whenever indicated.

Clinical Laboratory Investigations

Haematologic and blood chemistry measurements were carried out by standard methods on 32, respectively 33 randomised rats from the control and three concentration groups at the end of the treatment period.

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*) Y. Lepage, Biometrika (1971) 58: pp. 213-217

**) H. R. Jonckheere, Biometrika (1954) 41: pp. 133-145

Compound: TK 10'410

Observations and Records

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Compound: TK 10'410

To reduce the biologic variability due to circadian rhythms, blood sampling for haematology and blood chemistry was between the hours of 8.00 and 9.00 a.m. For blood chemistry measurements food was withheld for 18 hours prior to blood removal.

The site of blood removal was the orbital sinus and a micro-haematocrit glass capillary tube was used.

Blood samples from each animal with the respective anticoagulant (EDTA for performing the complete blood count, 3.8 % Sodium Citrate for coagulation testing and Heparin for blood chemistry measurements) were aliquoted into individual vials.

No anaesthesia was used to restrain the animals. All blood collection was by manual restraint only.

The quantitative assay of all blood parameters was completed within an 8 hr. period under "Quality Control" conditions.

The quality control systems used in haematology and blood chemistry were as follows:

Haematology Reference Control:	CH·60 Normal (Merz + Dade) CH·60 Abnormal " " 4C Normal (Coulter) 4C Abnormal " PLACHECK-100 (TOA Medical) IL 282 CO-OXIMETER Control (Instrumentation Lab. Inc.)
Coagulation Reference Control:	CI-TROL-1 (Merz + Dade) CI-TROL-2 " " CI-TROL-3 " " CONTROL PLASMA (Behringwerke) PATHOPLASMA-1 " PATHOPLASMA-2 "
Blood Chemistry Reference Control:	MONI·TROL I (Merz + Dade) MONI·TROL II " " ENZA·TROL SERONORM (Nyegaard) LEDER·NORM (Cyanamid) LEDER·TROL "

Parameter used in Haematologic, Blood Chemistry and Urinalysis Studies

	HAEMATOLOGY	METHOD	UNIT
☑	Haemoglobin (Hb)	Cyanmethaemoglobin. Coulter Haemoglobinometer	g/100 ml
☑	Methaemoglobin (MHb)	IL 282 CO-OXIMETER	%
☐	Carboxyhaemoglobin (COHb)	IL 282 CO-OXIMETER	%
☑	Erythrocytes (RBC)	Coulter Counter Model ZF	X 10^6 Cells/cmm
☑	Packed Cell Volume (PCV)	Clay-Adams Microhaematocrit Centrifuge. Centrifugation by 12,500 r.p.m./3 min.	Vol.-%
☑	Mean Corpuscular Volume (MCV)	Caculated value - $\frac{\text{PCV (\%) X 10}}{\text{RBC (X10}^6/\text{mm}^3)}$	cμ
☑	Mean Corpuscular Haemoglobin (MCH)	Calculated value - $\frac{\text{Hb (g \%) x 10}}{\text{RBC (X10}^6/\text{mm}^3)}$	μμg

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Reticulocytes:	Supravital staining with brilliant cresyl blue.	% Retics/1000 RBC
Inclusion Bodies (I.B.): (Heinz Bodies)	Supravital staining with neutral red and brilliant green. Examination of 8-10 fields if only a few Heinz Bodies were seen. When numerous 100 cells are counted and the rating made in accordance with the following scheme:	

% RBC showing Heinz Bodies	Evaluation
Negative	0
Occasional	1
1 - 10 %	2
11 - 40 %	3
41 - 75 %	4
76 -100 %	5

Thrombocytes:	Electronic counting with TOA Platelet Counter (Model PL-100)	x 10^3 cells/cmm
Prothrombin Time (P):	Quick's one-stage method using Coagulometer of Schnitger and Gross with plasma and Thromboplastin (Merz & Dade).	secs.
Activated Partial Thromboplastin Time (APTT)	Dade's activated Cephaloplastin method using Coagulometer of Schnitger and Gross: The partial thromboplastin time with kaolin, Proctor, R.R. & Rapaport, S.I., Amer. J. Clin. Path. 36:212 (1961).	secs.

00012

☐ **Plasma Viscosity:**	Harkness Viscometer. Readings made at 25°C to 0.02 sec. and compared with distilled water (relative viscosity = 1.0). "Rate of Shear" was 660 sec. $^{-1}$.	cp.
☑ **Leucocytes:**		
☑ **Total Count:**	Coulter Counter Model ZF	X 10^3 cells/cmm
☑ **Differential Count:**	Blood smear stained with the "Ames Hema-Tek Slide Stainer" using "Hema-Tek" Stain-Pak.	
	Meta-Myl. = Metamylocytes Band = Band Cells Seg = Segmented Neutrophils Ly = Lymphocytes Mo = Monocytes Eo = Eosinophils Ba = Basophils NBL/100 WBC = Normoblast	expressed as a % of total count

Blood Chemistry

☑ **Glucose:**	LKB Ultrolab System. UV-Glucose dehydrogenase method. Banauch, D. et al., Z. klin. Chem. Klin. Biochem. 13, 101-107 (1975).	mg/100 ml
☑ **Urea (Urea-N):**	LKB Ultrolab System. Urease method (Berthelot-Reaction). Fawcett, J.K. & Scott, J.E., J. Clin. Path. 13, 156 (1960).	mg/100 ml

Glutamate-Oxalacetate Transaminase (GOT):	Eppendorf KEA-5080. UV-absorption with NADH	mU/ml
Glutamate-Pyruvate Transaminase (GPT):	Eppendorf KEA-5080. UV-absorption with NADH	mU/ml
Lactate Dehydrogenase (LDH):	Eppendorf KEA-5080. UV-absorption with NADH	mU/ml
Alkaline Phosphatase (AP):	Eppendorf KEA-5080. Enzymatic hydrolysis of p-nitro-phenylphosphate. O.A. Bessey et al., J. Biol. Chem. 164, 321 (1946).	mU/ml
γ-Glutamyl Transpeptidase (γ-GT):	Eppendorf KEA-5080. γ-glutamyl-p-nitroanilide + glycylglycine. Szasz, G.: Clin. Chem., 15, 124 (1969).	mU/ml
Acetyl Cholinesterase (Erythrocyte, Plasma and Brain):	Colorimetric: Modified acetylthiocholine DTNB method. Voss, G. and Sachsse, K. Toxicol. Appl. Pharmacol. 16, 764 (1970).	Klett Units

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Total Bilirubin:	LKB Ultrolab System. Diazotization reaction in the presence of accelerator caffeine-sodium benzoate. Jedrassik, L. & Grof, P., Biochem. Ztschr. 297, 81 (1938).	mg/100 ml
Total Protein:	LKB Ultrolab System. Biuret reaction. Henry, R.J., Clinical Chemistry: Principles and Technics, Harper and Row Publishers, New York, (1964).	g/100 ml
Protein Electrophoresis:	Agarose gel electrophoresis utilizing Bioware's Cool Pak electrophoretic cell. Evaluation with the Helena Quick Scan and Quick Quant.	%
Electrolytes:		
Sodium (Na^+):	Flame Photometer (EEL 450)	mEq/l
Potassium (K^+):	Flame Photometer (EEL 450)	mEq/l
Cloride (Cl^-):	Coulometric-Amperometric titration (Buchler-Cotlove Chloridometer).	mEq/l

RESULTS

Food Consumption and Body Weight Gain

There was a reduction in food consumption in the highest concentration group during the first 15 days of the exposure period. In the intermediate group it was reduced during the first 8 days of exposure, whereas in the low dose group it was reduced in males during days 3 to 8 only.

The body weights of the male rats of groups 3 and 4 were significantly ($p = 0.01$) decreased during the entire exposure period, whereas those of the male rats of group 2 were partially decreased ($p = 0.01$) at days 8, 9 and 10 of the test.

The body weights of the female rats of group 3 were significantly ($p = 0.01$) decreased at days 3 to 10 and 14 and 15 of the exposure period, those of the females of group 4 during days 2 to 10, whereas the female animals of group 2 only showed a significant decrease at day 9 of the exposure period.

During the recovery period the food intake and the body weight gain of the rats of group 3 was again comparable to that of the controls.

Toxic Symptoms

At the beginning of the exposure period the rats of all concentration groups showed dyspnoe, exophthalmos and ruffled fur. In addition the animals of group 4 showed lateral or ventral position and bloating of the abdomen.

During the observation period the rats of group 3 showed no toxic symptoms.

15 rats (6 males, 9 females) of group 4 died spontaneously during the exposure period.

Eye Examination

No ocular changes were seen.

Compound: TK 10'410

Droplet Size Distribution

Droplet size distribution analysis of the chamber airborne droplets showed that 80 respectively 90 % were smaller than 7 micron in diameter (page 19).

Temperature and Relative Humidity (test chamber)

The values for the above mentioned parameters measured in the test chambers during the test period are shown in the table below:

Days	Group 1		Group 2		Group 3		Group 4	
	Humid. % RH	Temp. °C	Humid. % RH	Temp. °C	Humid. % RH	Temp. °C	Humid. % RH	Temp. °C
1	50	24	54	24	50	24	46	24
2	50	26	48	26	52	26	50	26
3	50	26	50	26	58	26	52	26
6	50	26	50	26	38	28	32	26
7	50	24	52	24	48	24	40	24
8	50	24	48	24	44	24	42	26
9	58	22	40	22	44	22	26	24
10	58	24	50	26	50	26	32	24
13	54	26	44	26	48	26	30	26
14	50	26	48	26	50	26	32	24
15	54	26	48	26	50	26	30	24
16	52	24	52	26	50	24	30	22
17	50	24	50	26	50	26	30	26
20	50	26	50	26	50	26	30	24
21	48	26	44	26	56	26	28	26
$\bar{x}\pm SD$	51/3.1	25/1.2	48/3.5	25/1.2	49/4.7	25/1.4	35/8.4	25/1.3

Compound: TK 10'410

Clinical Laboratory Investigations

Haematology:

The observed haematological findings between treated rats and controls were generally unremarkable.

A slight increase in the haemoglobin concentration, packed cell volume and erythrocyte count was observed in the rats at the 305 mg/m³ concentration level. This is attributed to a slight degree of exsiccosis.

At the same concentration level a moderate shift in the differential leucocyte count occurred, i.e. a decrease in the percentage of polymorphonuclear neutrophils and increase in the percentage of lymphocytes.

Blood Chemistry:

In the assessment of blood chemistry values the findings were generally unremarkable and comparable to those of the controls.

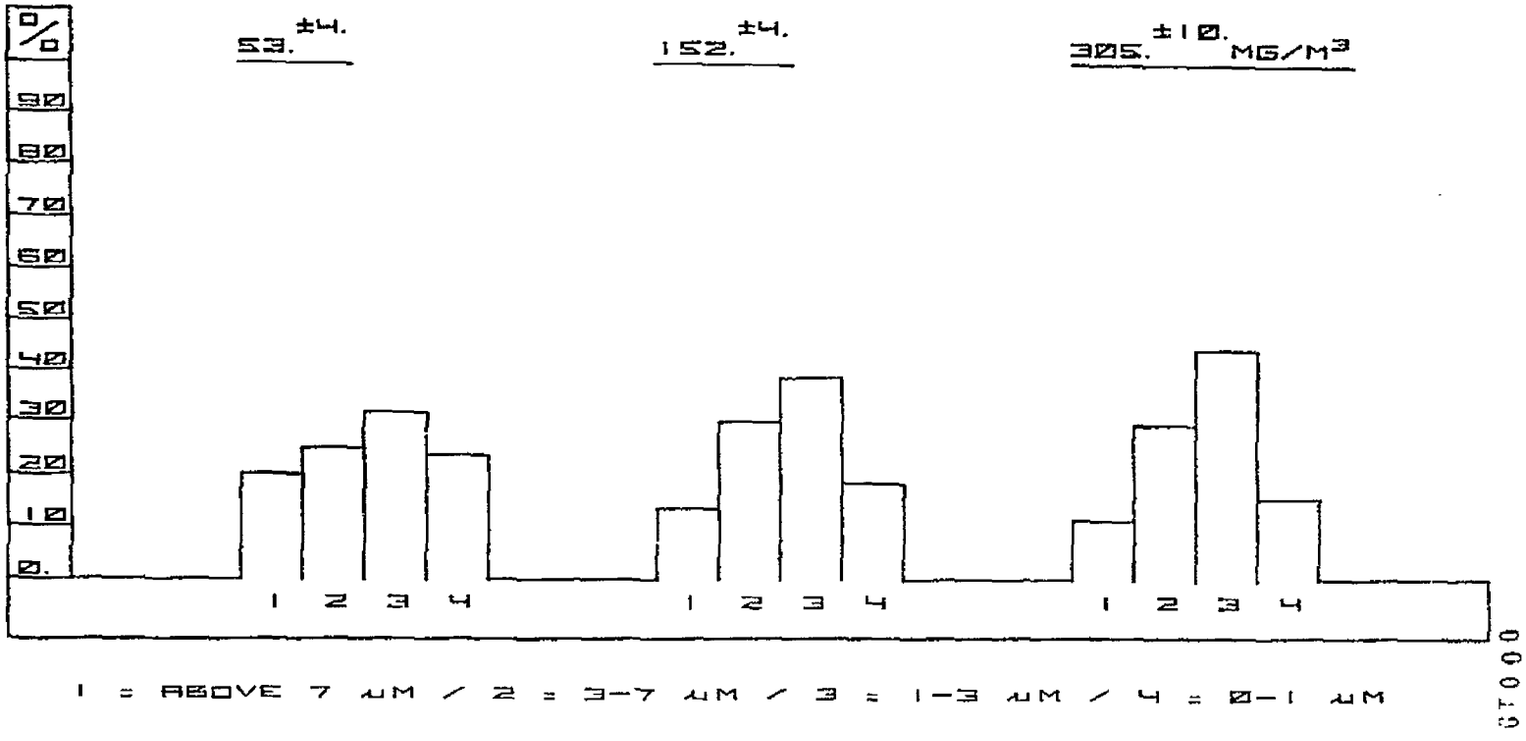
An increase in the BUN, GOT, GPT and LDH level was observed in two out of three male rats (36 M and 37 M) at the 305 mg/m³ concentration level. Proportional changes in the electropherogram, i.e. a relative decrease of the alpha-1 and beta-2 globulin fractions was also observed in the animals at this level.

EXP.6432.

TK 10410

FIG. 1.

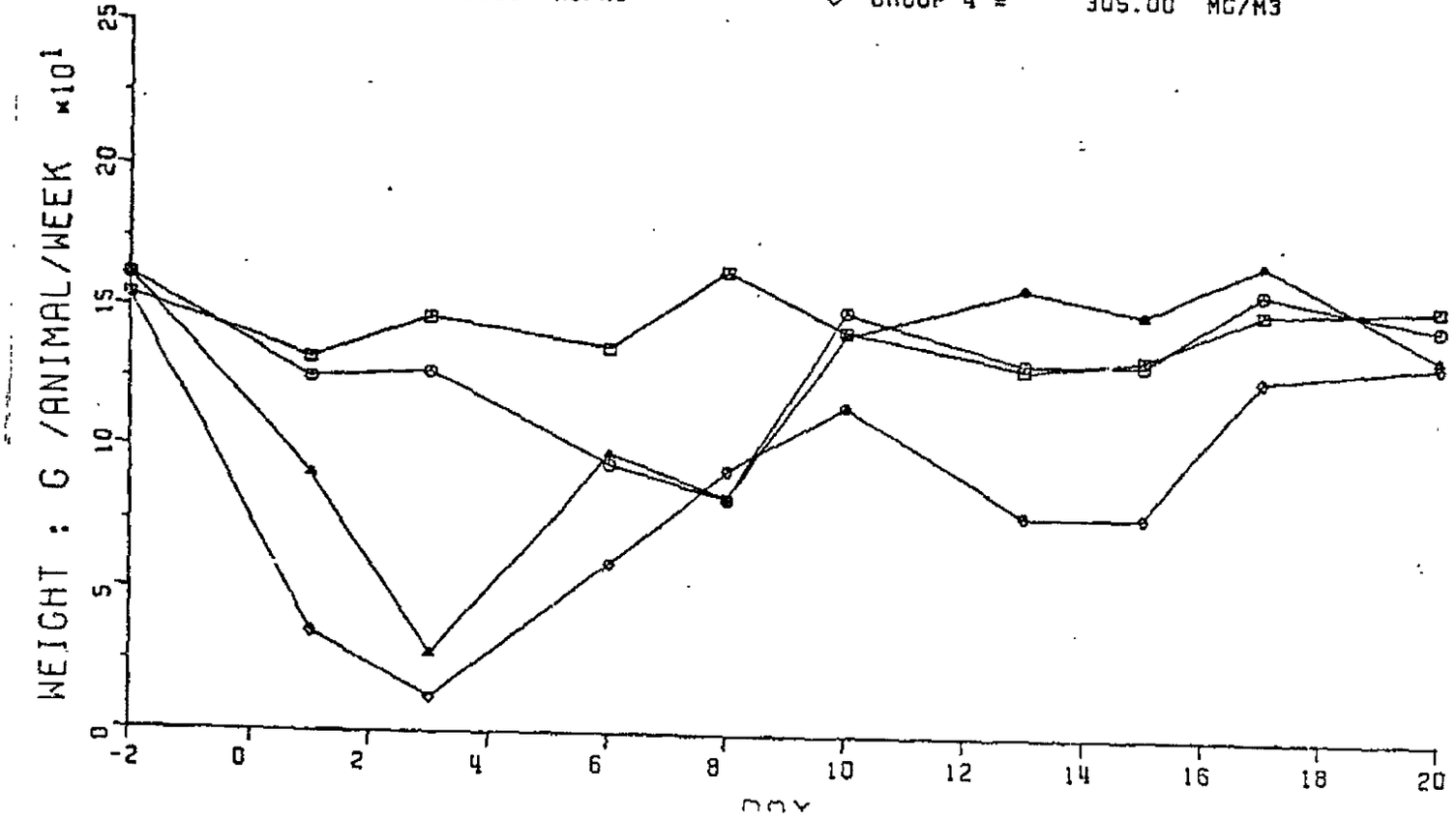
MEAN PARTICLE SIZE DISTRIBUTION
CASCADE IMPACTOR
AIR FLOW RATE 17.5 L/MIN



M E A N F O O D C O N S U M P T I O N

SPEC.: RAT RAI (=SIV-50) EXP. : R06432/1
SEX .: MALE COMP.: TK 10410

- GROUP 1 = 0.00 MG/M3
- GROUP 2 = 53.00 MG/M3
- GROUP 3 = 152.00 MG/M3
- GROUP 4 = 305.00 MG/M3

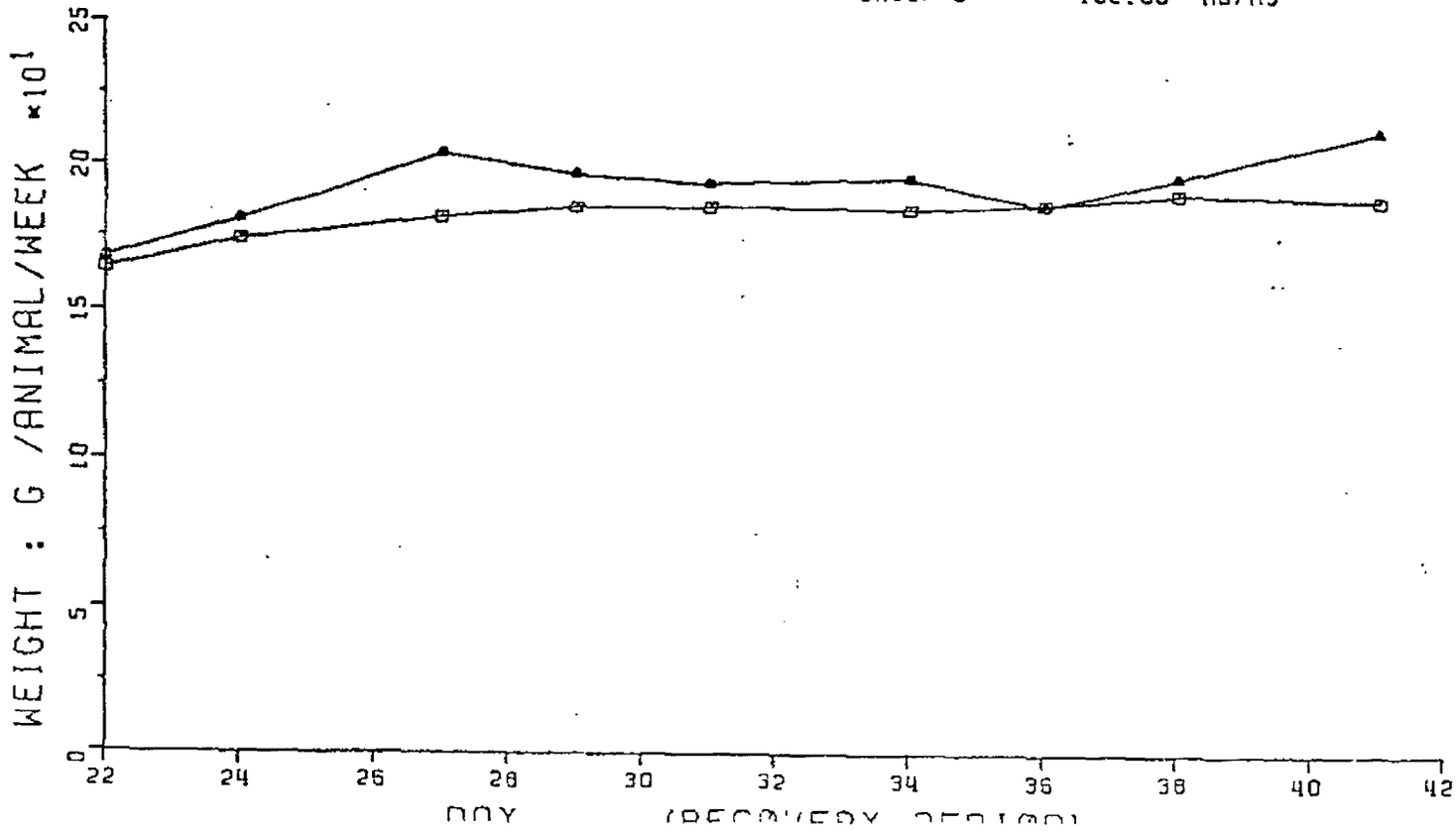


02000

M E A N F O O D C O N S U M P T I O N

SPEC.: RAT RAI (=SIV-50) EXP. : R06432/1
SEX .: MALE COMP.: TK 10410

□ GROUP 1 = 0.00 MG/M3 △ GROUP 3 = 152.00 MG/M3

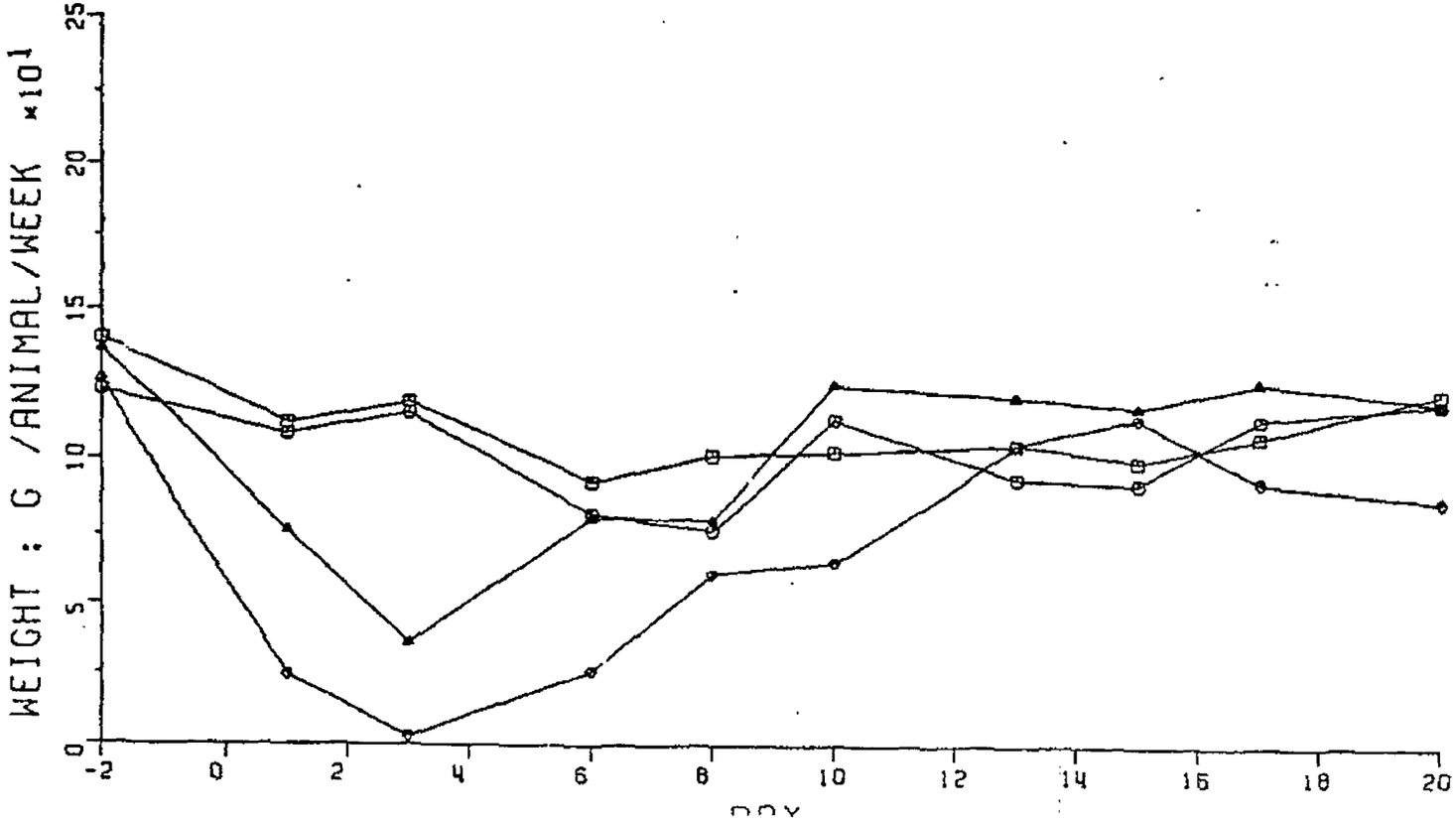


T2000

M E A N F O O D C O N S U M P T I O N

SPEC.:RAT RAI (=SIV-50) EXP. :R06432/1
SEX .:FEMALE COMP.:TK 10410

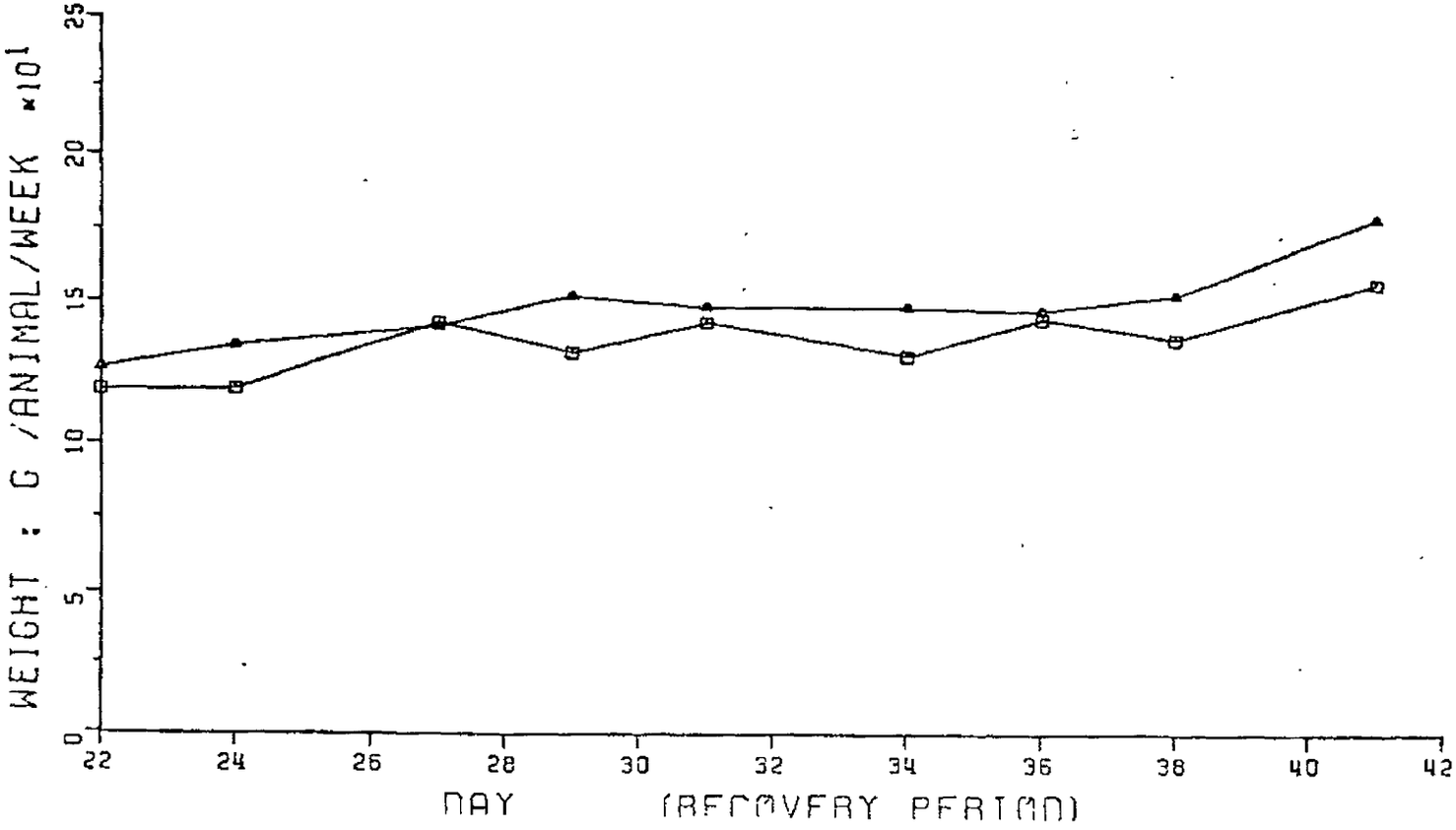
⊡ GROUP 1 = 0.00 MG/M3 ▲ GROUP 3 = 152.00 MG/M3
⊙ GROUP 2 = 53.00 MG/M3 ◇ GROUP 4 = 305.00 MG/M3



M E A N F O O D C O N S U M P T I O N

SPEC.:RAT RAI (=SIV-50) EXP. :R06432/1
SEX .:FEMALE COMP.:TK 10410

□ GROUP 1 = 0.00 MG/M3 △ GROUP 3 = 152.00 MG/M3



2000

MEAN FOOD CONSUMPTION

G / ANIMAL / WEEK

SPECIES : RAT RAI (=SIV-50)
SEX : MALE

EXP. NO. : R06432/1
COMPOUND : TK 10410

DAY	DOSE IN MG/M3 0.000 NO	0.000 MEAN	53.000 NO	53.000 MEAN	152.000 NO	152.000 MEAN	305.000 NO	305.000 MEAN	TREND
-2	10	154	10	161	10	161	10	154	
1	10	133	10	126	10	91	10	35	<--
3	10	147	10	128	10	27	10	12	<--
6	10	137	10	95	10	99	9	61	
8	10	165	10	84	10	84	7	93	
10	10	143	10	151	10	142	6	117	
13	10	131	10	133	10	159	6	79	
15	10	135	10	133	10	151	6	79	
17	10	152	10	159	10	169	4	128	
20	10	154	10	147	10	137	4	134	

NO = NO. OF LIVING ANIMALS / GROUP (END OF THE WEEK)
*** NO STATISTICALLY SIGN. DIFFERENCE BETWEEN CONTROL AND TREATED GROUPS (SIGN.L.= 0.010)
<-- = SIGN. NEG. TREND FROM CONTROL TO HIGHEST DOSAGE-GROUP (SIGN.L.= 0.010)

000124

MEAN FOOD CONSUMPTION

G / ANIMAL / WEEK

SPECIES : RAT RAI (=SIV-50)
SEX : MALE

EXP. NO. : R06432/1
COMPOUND : TK 10410

RECOVERY PERIOD

DAY	DOSE IN MG/M3 0.000 NO	0.000 MEAN	53.000 NO	53.000 MEAN	152.000 NO	152.000 MEAN	305.000 NO	305.000 MEAN	TREND
22	5	165			5	168			
24	5	175			5	181			
27	5	182			5	204			
29	5	186			5	197			
31	5	186			5	194			
34	5	185			5	196			
36	5	187			5	186			
38	5	191			5	196			
41	5	189			5	213			

NO = NO. OF LIVING ANIMALS / GROUP (END OF THE WEEK)
*** NO STATISTICALLY SIGN. DIFFERENCE BETWEEN CONTROL AND TREATED GROUPS (SIGN.L.= 0.010)
*** NO STATISTICALLY SIGN. TRENDS (SIGN.L.= 0.010)

52000

MEAN FOOD CONSUMPTION

G / ANIMAL / WEEK

SPECIES : RAT RAI (=SIV-50)
SEX : FEMALE

EXP. NO. : R06432/1
COMPOUND : TK 10410

DAY	DOSE IN MG/M3 0.000 NO	0.000 MEAN	53.000 NO	53.000 MEAN	152.000 NO	152.000 MEAN	305.000 NO	305.000 MEAN	TREND
-2	10	140	10	123	10	137	10	126	
1	10	112	10	109	10	77	10	25	
3	10	119	10	115	10	36	10	2	<--
6	10	93	10	83	10	81	8	26	
8	10	102	10	77	10	81	4	61	
10	10	103	10	113	10	125	2	66	
13	10	105	10	95	10	121	2	105	
15	10	100	10	93	10	117	2	114	
17	10	107	10	113	10	126	2	93	
20	10	123	10	119	10	119	2	88	

NO = NO. OF LIVING ANIMALS / GROUP (END OF THE WEEK)
*** NO STATISTICALLY SIGN. DIFFERENCE BETWEEN CONTROL AND TREATED GROUPS (SIGN.L.= 0.010)
<-- = SIGN. NEG. TREND FROM CONTROL TO HIGHEST DOSAGE-GROUP (SIGN.L.= 0.010)

MEAN FOOD CONSUMPTION

G / ANIMAL / WEEK

SPECIES : RAT RAI (=SIV-50)
SEX : FEMALE

EXP. NO. : R06432/1
COMPOUND : TK 10410

RECOVERY PERIOD

DAY	DOSE IN MG/M3 0.000 NO	0.000 MEAN	53.000 NO	53.000 MEAN	152.000 NO	152.000 MEAN	305.000 NO	305.000 MEAN	TREND
22	5	119			5	126			
24	5	119			5	134			
27	5	142			5	141			
29	5	132			5	152			
31	5	142			5	148			
34	5	130			5	148			
36	5	144			5	146			
38	5	136			5	152			
41	5	157			5	179			

NO = NO. OF LIVING ANIMALS / GROUP (END OF THE WEEK)
*** NO STATISTICALLY SIGN. DIFFERENCE BETWEEN CONTROL AND TREATED GROUPS (SIGN.L.= 0.010)
*** NO STATISTICALLY SIGN. TRENDS (SIGN.L.= 0.010)

42000

INDIVIDUAL FOOD CONSUMPTION

G / ANIMAL / WEEK

SPECIES : RAT RAI (=SIV-50)
SEX : MALE
GROUP : 1 = 0.000 MG/M3

EXP. NO. : R06432/1
COMPOUND : TK 10410

CAGE NO.	DAY -2		1		3		6		8		10		13		15		17		20	
	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL
1	5	154	5	140	5	149	5	145	5	133	5	149	5	130	5	137	5	154	5	154
2	5	154	5	126	5	145	5	129	5	196	5	138	5	133	5	133	5	149	5	154

NO = NO. OF LIVING ANIMALS / CAGE (END OF THE WEEK)

GROUP : 2 = 53.000 MG/M3

CAGE NO.	DAY -2		1		3		6		8		10		13		15		17		20	
	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL
3	5	161	5	119	5	140	5	103	5	98	5	147	5	126	5	126	5	159	5	147
4	5	161	5	133	5	117	5	88	5	70	5	154	5	140	5	140	5	159	5	147

NO = NO. OF LIVING ANIMALS / CAGE (END OF THE WEEK)

GROUP : 3 = 152.000 MG/M3

CAGE NO.	DAY -2		1		3		6		8		10		13		15		17		20	
	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL
5	5	161	5	105	5	27	5	113	5	84	5	152	5	165	5	161	5	175	5	147
6	5	161	5	77	5	28	5	84	5	84	5	133	5	154	5	140	5	163	5	126

NO = NO. OF LIVING ANIMALS / CAGE (END OF THE WEEK)

02000

INDIVIDUAL FOOD CONSUMPTION

G / ANIMAL / WEEK

SPECIES : RAT RAI (=SIV-50)
SEX : MALE
GROUP : 4 = 305.000 MG/M3

EXP. NO. : R06432/1
COMPOUND : TK 10410

CAGE NO.	DAY -2		1		3		6		8		10		13		15		17		20	
	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL
7	5	154	5	35	5	12	4	56	3	93	2	88	2	70	2	70	1	140	1	140
8	5	154	5	35	5	12	5	65	4	93	4	146	4	88	4	88	3	117	3	128

NO = NO. OF LIVING ANIMALS / CAGE (END OF THE WEEK)

INDIVIDUAL FOOD CONSUMPTION

G / ANIMAL / WEEK

SPECIES : RAT RAI (=SIV-50)
SEX : FEMALE
GROUP : 1 = 0.000 MG/M3

EXP. NO. : R06432/1
COMPOUND : TK 10410

CAGE NO.	DAY -2		1		3		6		8		10		13		15		17		20	
	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL
9	5	140	5	112	5	126	5	81	5	105	5	103	5	109	5	98	5	114	5	133
10	5	140	5	112	5	112	5	105	5	98	5	103	5	102	5	102	5	100	5	112

NO = NO. OF LIVING ANIMALS / CAGE (END OF THE WEEK)

GROUP : 2 = 53.000 MG/M3

CAGE NO.	DAY -2		1		3		6		8		10		13		15		17		20	
	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL
11	5	112	5	98	5	110	5	79	5	77	5	103	5	91	5	84	5	105	5	112
12	5	133	5	119	5	121	5	87	5	77	5	124	5	98	5	102	5	121	5	126

NO = NO. OF LIVING ANIMALS / CAGE (END OF THE WEEK)

GROUP : 3 = 152.000 MG/M3

CAGE NO.	DAY -2		1		3		6		8		10		13		15		17		20	
	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL
13	5	133	5	77	5	42	5	76	5	77	5	124	5	123	5	123	5	126	5	119
14	5	140	5	77	5	30	5	86	5	84	5	126	5	119	5	112	5	126	5	119

NO = NO. OF LIVING ANIMALS / CAGE (END OF THE WEEK)

INDIVIDUAL FOOD CONSUMPTION

G / ANIMAL / WEEK

SPECIES : RAT RAI (=SIV-50)
SEX : FEMALE
GROUP : 4 = 305.000 MG/M3

EXP. NO. : R06432/1
COMPOUND : TK 10410

CAGE NO.	DAY -2		1		3		6		8		10		13		15		17		20	
	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL
15	5	126	5	28	5	2	5	21	4	61	2	66	2	105	2	114	2	93	2	88
16	5	126	5	21	5	2	3	30												

NO = NO. OF LIVING ANIMALS / CAGE (END OF THE WEEK)

INDIVIDUAL FOOD CONSUMPTION

G / ANIMAL / WEEK

SPECIES : RAT RAI (=SIV-50)
SEX : MALE
GROUP : 1 = 0.000 MG/M3

EXP. NO. : R06432/1
COMPOUND : TK 1G410

RECOVERY PERIOD

CAGE NO.	DAY 22		24		27		29		31		34		36		38		41	
	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL
1	5	165	5	175	5	182	5	186	5	186	5	185	5	187	5	191	5	189

NO = NO. OF LIVING ANIMALS / CAGE (END OF THE WEEK)

GROUP : 3 = 152.000 MG/M3

RECOVERY PERIOD

CAGE NO.	DAY 22		24		27		29		31		34		36		38		41	
	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL
5	5	168	5	181	5	204	5	197	5	194	5	196	5	186	5	196	5	213

NO = NO. OF LIVING ANIMALS / CAGE (END OF THE WEEK)

I N D I V I D U A L F O O D C O N S U M P T I O N

G / ANIMAL / WEEK

SPECIES : RAT RAI (=SIV-50)
SEX : FEMALE
GROUP : 1 = 0.000 MG/M3

EXP. NO. : R06432/1
COMPOUND : TK 10410

RECOVERY PERIOD

CAGE NO.	DAY 22		24		27		29		31		34		36		38		41	
	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL
9	5	119	5	119	5	142	5	132	5	142	5	130	5	144	5	136	5	157

NO = NO. OF LIVING ANIMALS / CAGE (END OF THE WEEK)

GROUP : 3 = 152.000 MG/M3

RECOVERY PERIOD

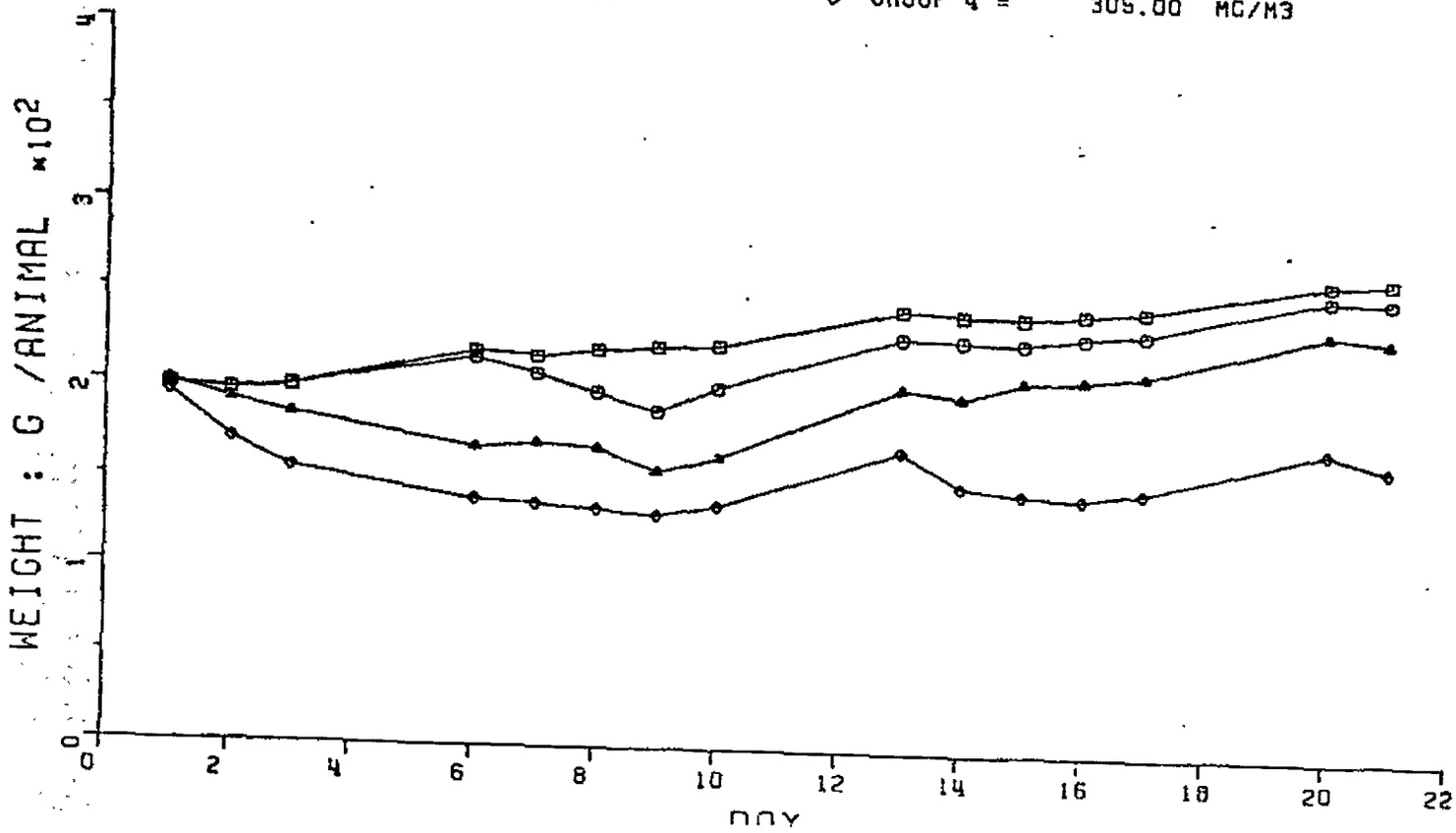
CAGE NO.	DAY 22		24		27		29		31		34		36		38		41	
	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL	NO	TOTAL
13	5	126	5	134	5	141	5	152	5	148	5	148	5	146	5	152	5	179

NO = NO. OF LIVING ANIMALS / CAGE (END OF THE WEEK)

MEAN BODY WEIGHT

SPEC.:RAT RAI (=SIV-50) EXP. :R06432/1
SEX .:MALE COMP.:TK 10410

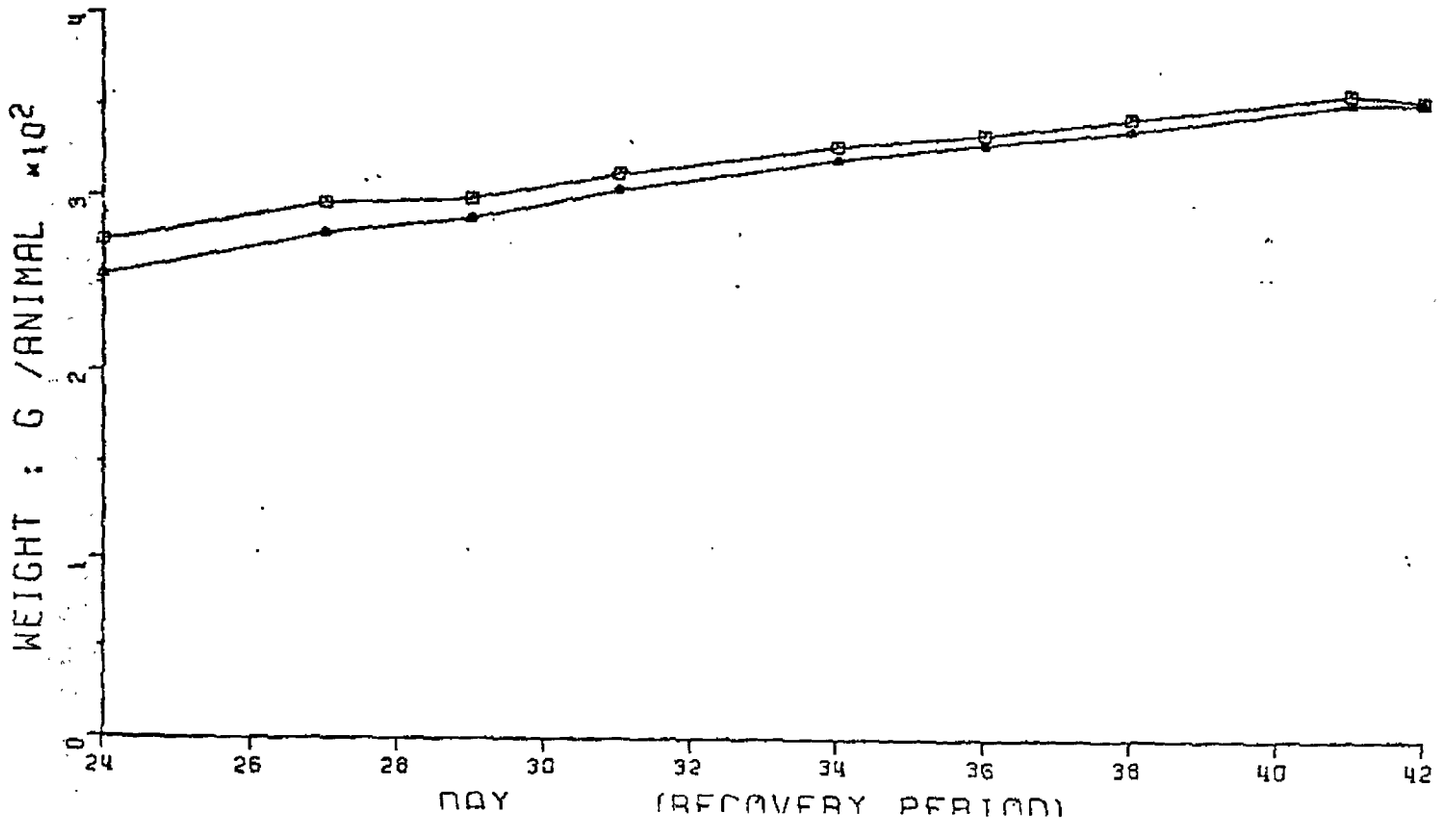
□ GROUP 1 = 0.00 MG/M3
○ GROUP 2 = 53.00 MG/M3
△ GROUP 3 = 152.00 MG/M3
◇ GROUP 4 = 305.00 MG/M3



M E A N B O D Y W E I G H T

SPEC.:RAT RAI (=SIV-50) EXP. :R06432/1
SEX .:MALE COMP.:TK 10410

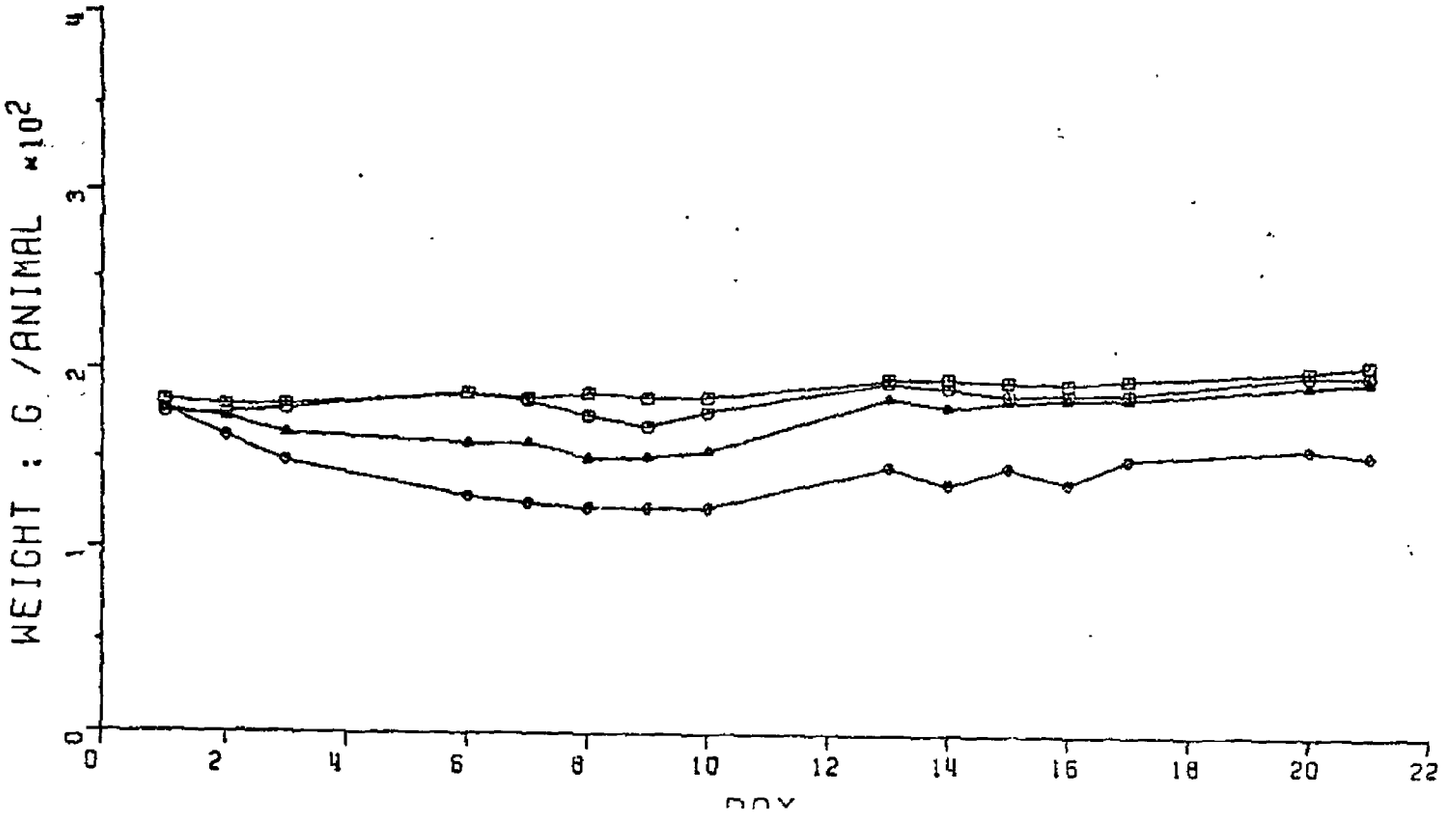
□ GROUP 1 = 0.00 MG/M3 ▲ GROUP 3 = 152.00 MG/M3



M E A N B O D Y W E I G H T

SPEC.:RAT RAI (=SIV-50) EXP. :R06432/1
SEX .:FEMALE COMP.:TK 10410

⊡ GROUP 1 = 0.00 MG/M3 ▲ GROUP 3 = 152.00 MG/M3
⊙ GROUP 2 = 53.00 MG/M3 ◇ GROUP 4 = 305.00 MG/M3



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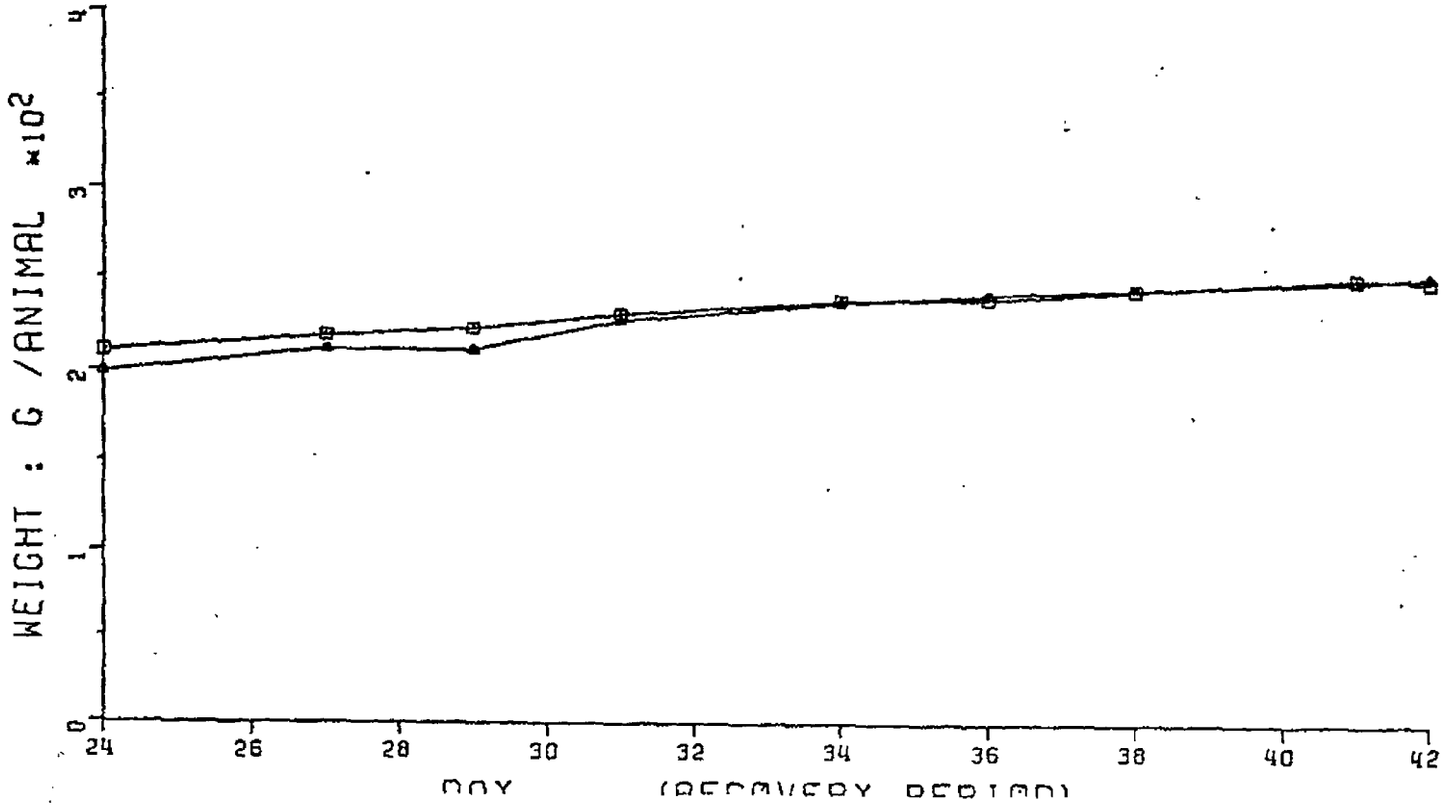
M E A N B O D Y W E I G H T

SPEC.:RAT RAI (=SIV-50) EXP. :R06432/1
SEX .:FEMALE COMP.:TK 10410

□ GROUP 1 = 0.00 MG/M3 ▲ GROUP 3 = 152.00 MG/M3



MEAN BODY WEIGHT

G / ANIMAL

SPECIES : RAT RAI (=SIV-50)
SEX : MALE

EXP. NO. : R06432/1
COMPOUND : TK 10410

DAY	DOSE IN MG/M3 0.000		53.000		152.000		305.000		TREND
	NO	MEAN	NO	MEAN	NO	MEAN	NO	MEAN	
1	10	199	10	199	10	200	10	195	
2	10	197	10	197	10	192	10	171 *	<--
3	10	199	10	199	10	185 *	10	157 *	<--
6	10	220	10	215	10	168 *	10	140 *	<--
7	10	217	10	207	10	171 *	9	138 *	<--
8	10	221	10	198 *	10	168 *	9	135 *	<--
9	10	223	10	198 *	10	157 *	7	132 *	<--
10	10	225	10	201 *	10	165 *	7	138 *	<--
13	10	244	10	230	10	202 *	6	170 *	<--
14	10	242	10	229	10	198 *	6	151 *	<--
15	10	242	10	228	10	207 *	6	147 *	<--
16	10	244	10	232	10	208 *	6	146 *	<--
17	10	247	10	234	10	212 *	6	150 *	<--
20	10	264	10	255	10	237 *	4	174 *	<--
21	10	266	10	254	10	233 *	4	165 *	<--

NO = NO. OF LIVING ANIMALS / GROUP
* = SIGN. DIFFERENCE (LOCATION AND/OR DISPERSION) BETWEEN CONTROL (GROUP 1) AND GROUP X (SIGN.L.= 0.010)
<-- = SIGN. NEG. TREND FROM CONTROL TO HIGHEST DOSAGE-GROUP (SIGN.L.= 0.010)

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MEAN BODY WEIGHT

G / ANIMAL

EXP. NO. : R06432/1
COMPOUND : TK 10410

SPECIES : RAT RAI (=SIV-50)
SEX : MALE

RECOVERY PERIOD

DAY	DOSE IN MG/M3 0.000 NO	0.000 MEAN	53.000 NO	53.000 MEAN	152.000 NO	152.000 MEAN	305.000 NO	305.000 MEAN	TREND
24	5	275			5	255			
27	5	297			5	280			
29	5	300			5	288			
31	5	314			5	305			
34	5	329			5	321			
36	5	336			5	330			
38	5	345			5	338			
41	5	358			5	353			
42	5	355			5	353			

NO = NO. OF LIVING ANIMALS / GROUP
*** NO STATISTICALLY SIGN. DIFFERENCE BETWEEN CONTROL AND TREATED GROUPS (SIGN.L.= 0.010)
*** NO STATISTICALLY SIGN. TRENDS (SIGN.L.= 0.010)

MEAN BODY WEIGHT

G / ANIMAL

SPECIES : RAT RAI (=SIV-50)
SEX : FEMALE

EXP. NO. : R06432/1
COMPOUND : TK 10410

DAY	DOSE IN MG/M3 0.000 NO	0.000 MEAN	53.000 NO	53.000 MEAN	152.000 NO	152.000 MEAN	305.000 NO	305.000 MEAN	TREND
1	10	183	10	176	10	177	10	178	
2	10	180	10	175	10	173	10	162 *	<--
3	10	181	10	178	10	164 *	10	149 *	<--
6	10	188	10	188	10	159 *	10	129 *	<--
7	10	185	10	183	10	161 *	8	125 *	<--
8	10	188	10	175	10	151 *	7	122 *	<--
9	10	185	10	169 *	10	151 *	4	123 *	<--
10	10	185	10	177	10	155 *	4	123 *	<--
13	10	196	10	194	10	185	2	146	<--
14	10	196	10	192	10	180 *	2	136	<--
15	10	195	10	187	10	184 *	2	146	<--
16	10	194	10	188	10	185	2	138	<--
17	10	197	10	188	10	186	2	151	<--
20	10	202	10	199	10	193	2	158	
21	10	205	10	199	10	196	2	154	<--

NO = NO. OF LIVING ANIMALS / GROUP
* = SIGN. DIFFERENCE (LOCATION AND/OR DISPERSION) BETWEEN CONTROL (GROUP 1) AND GROUP X (SIGN.L.= 0.010)
<-- = SIGN. NEG. TREND FROM CONTROL TO HIGHEST DOSAGE-GROUP (SIGN.L.= 0.010)

MEAN BODY WEIGHT

G / ANIMAL

SPECIES : RAT RAI (=SIV-50)
SEX : FEMALE

EXP. NO. : R06432/1
COMPOUND : TK 10410

RECOVERY PERIOD

DAY	DOSE IN MG/M3 0.000 NO	0.000 MEAN	53.000 NO	53.000 MEAN	152.000 NO	152.000 MEAN	305.000 NO	305.000 MEAN	TREND
24	5	210			5	199			
27	5	219			5	211			
29	5	223			5	211			
31	5	231			5	227			
34	5	238			5	236			
36	5	239			5	241			
38	5	244			5	245			
41	5	251			5	249			
42	5	249			5	252			

NO = NO. OF LIVING ANIMALS / GROUP
*** NO STATISTICALLY SIGN. DIFFERENCE BETWEEN CONTROL AND TREATED GROUPS (SIGN.L.= 0.010)
*** NO STATISTICALLY SIGN. TRENDS (SIGN.L.= 0.010)

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INDIVIDUAL BODY WEIGHT

G / ANIMAL

SPECIES : RAT RAI (=SIV-50)
SEX : MALE
GROUP : 1 = 0.000 MG/M3

EXP. NO. : R06432/1
COMPOUND : TK 10410

ANIMAL NO.	DAY 1	2	3	6	7	8	9	10	13	14	15	16	17	20	21
1	212	207	208	229	223	230	233	230	257	255	262	261	259	270	270
2	197	197	202	224	223	230	240	237	256	256	249	251	262	279	284
3	197	190	193	216	213	218	215	212	233	233	229	233	233	253	253
4	203	201	203	225	223	228	230	235	256	255	248	252	256	278	280
5	200	200	205	219	218	220	221	220	233	233	232	235	235	248	249
6	194	194	194	221	220	226	230	236	250	255	254	258	260	280	276
7	197	194	200	214	210	210	210	212	238	234	237	239	245	257	261
8	186	183	183	204	200	198	203	210	227	223	228	229	235	248	251
9	202	200	200	226	222	228	230	236	256	256	257	262	264	286	284
10	202	199	203	220	216	218	220	220	233	224	220	222	218	245	248

GROUP : 2 = 53.000 MG/M3

ANIMAL NO.	DAY 1	2	3	6	7	8	9	10	13	14	15	16	17	20	21
11	195	190	193	212	200	200	200	206	227	223	224	228	229	248	251
12	198	193	194	208	200	195	190	206	229	226	223	230	232	261	259
13	208	203	200	221	212	200	193	213	240	244	232	232	238	261	260
14	188	188	191	213	204	200	183	193	232	234	234	236	238	259	259
15	202	197	202	229	220	220	216	220	241	240	240	242	241	265	260
16	185	185	192	210	204	195	190	200	224	226	227	233	235	252	251
17	196	193	196	200	194	177	160	180	212	209	213	215	222	240	240
18	207	206	206	219	214	193	185	200	228	227	222	233	233	250	245
19	203	198	197	217	210	193	175	195	226	224	227	224	227	245	247
20	207	213	216	225	214	205	192	200	240	240	240	245	246	271	272

57040

INDIVIDUAL BODY WEIGHT

G / ANIMAL

SPECIES : RAT RAI (=SIV-50)
SEX : MALE
GROUP : 3 = 152.000 MG/M3

EXP. NO. : RO6432/1
COMPOUND : TK 10410

ANIMAL NO.	DAY 1	2	3	6	7	8	9	10	13	14	15	16	17	20	21
21	203	197	194	176	186	181	170	177	212	210	214	219	221	243	244
22	210	202	184	180	180	187	160	172	204	200	216	216	224	248	242
23	198	196	190	183	170	175	170	170	217	206	214	210	218	244	239
24	205	196	190	160	170	172	164	174	210	212	214	226	228	248	249
25	203	195	191	168	180	172	152	164	206	201	213	214	220	240	241
26	203	185	175	164	157	152	140	150	187	181	198	194	202	229	223
27	200	190	175	151	167	160	145	152	187	178	190	192	193	223	215
28	208	194	193	168	157	164	160	160	198	197	206	209	208	238	232
29	189	178	173	156	167	164	150	160	197	193	201	202	202	226	222
30	186	182	185	177	177	157	160	170	206	202	205	202	205	226	223

GROUP : 4 = 305.000 MG/M3

ANIMAL NO.	DAY 1	2	3	6	7	8	9	10	13	14	15	16	17	20	21
31	197	165	154	124											
32	199	174	165	145	137	130	142	125	137	124	117	115	110		
33	184	175	163	153	139	127									
34	183	159	151	129	139	134	124	141	166	143	142	143	149	171	168
35	197	167	150	132	134	130	122	120							
36	191	169	155	146	134	144	126	123	140	130	143	133	159	182	154
37	196	174	165	140	136	136	123	138	177	150	137	145	138	159	151
38	185	158	139	123	116	116									
39	206	186	139	154	146	151	133	150	187	170	165	165	158	183	185
40	214	186	186	153	160	150	157	173	211	189	180	172	184		

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INDIVIDUAL BODY WEIGHT

G / ANIMAL

SPECIES : RAT RAI (=SIV-50)
SEX : FEMALE
GROUP : 1 = 0.000 MG/M3

EXP. NO. : R06432/1
COMPOUND : TK 10410

ANIMAL NO.	DAY 1	2	3	6	7	8	9	10	13	14	15	16	17	20	21
41	190	187	187	201	195	198	200	196	202	202	201	205	206	216	218
42	187	183	188	192	191	194	182	190	191	198	198	197	193	205	205
43	176	171	172	177	170	174	174	175	192	190	189	191	192	199	200
44	186	183	180	195	190	190	185	190	197	197	199	188	198	199	209
45	190	181	185	193	193	190	190	190	208	207	198	204	206	215	216
46	188	185	183	186	186	190	185	188	200	200	191	195	202	206	208
47	179	182	182	188	186	193	190	184	198	192	198	201	203	213	212
48	179	183	182	183	180	183	180	180	190	190	189	189	189	188	189
49	176	174	179	182	176	180	184	180	186	188	188	178	184	182	192
50	174	174	174	180	180	185	180	180	196	196	195	190	194	192	200

GROUP : 2 = 53.000 MG/M3

ANIMAL NO.	DAY 1	2	3	6	7	8	9	10	13	14	15	16	17	20	21
51	164	167	170	174	164	157	156	166	180	181	175	170	172	185	183
52	161	163	168	184	174	170	170	170	178	179	178	176	177	182	188
53	167	167	167	172	168	164	160	168	181	179	173	176	176	183	183
54	181	182	185	203	193	180	170	185	202	199	193	193	196	207	206
55	171	169	172	178	170	165	166	170	191	189	183	185	182	188	193
56	165	164	164	194	190	170	166	180	200	198	196	200	197	211	210
57	181	179	180	190	190	185	170	180	203	198	190	193	195	209	208
58	182	177	181	184	184	175	170	176	188	185	187	186	186	199	194
59	187	186	190	203	200	195	190	193	209	203	198	200	203	215	214
60	184	179	184	199	200	188	170	181	211	204	198	200	200	211	210

INDIVIDUAL BODY WEIGHT

G / ANIMAL

SPECIES : RAT RAI (=SIV-50)
SEX : FEMALE
GROUP : 3 = 152.000 MG/M3

EXP. NO. : R06432/1
COMPOUND : TK 10410

ANIMAL NO.	DAY 1	2	3	6	7	8	9	10	13	14	15	16	17	20	21
61	170	170	153	145	137	130	134	130	168	163	174	174	175	193	190
62	183	177	173	179	177	166	170	170	204	198	190	193	196	201	201
63	174	173	159	154	152	150	140	143	179	175	178	182	182	187	190
64	176	171	169	176	175	165	170	170	187	182	186	188	190	192	193
65	179	172	159	151	150	135	140	155	188	182	188	192	190	197	200
66	170	175	168	159	170	170	175	180	205	195	198	202	200	214	213
67	176	168	167	159	170	160	165	170	185	183	183	180	181	184	191
68	181	178	173	176	175	153	160	160	194	186	187	190	191	193	200
69	187	173	159	142	140	140	130	146	175	171	180	177	177	190	191
70	161	176	162	153	150	140	130	130	169	164	175	174	176	183	187

GROUP : 4 = 305.000 MG/M3

ANIMAL NO.	DAY 1	2	3	6	7	8	9	10	13	14	15	16	17	20	21
71	191	179	163	143	142	146	135	145	147	135	150	139	160	162	152
72	171	150	137	121	114	108									
73	170	168	150	127	118	110	115	107							
74	188	167	153	130	124	117	110	106							
75	173	157	145	130	130	130	130	132	145	137	142	136	142	153	156
76	185	168	159	137	135	134									
77	172	155	144	116											
78	172	158	140	124	117	110									
79	183	161	150	135											
80	180	161	149	125	119										

23000

INDIVIDUAL BODY WEIGHT

G / ANIMAL

SPECIES : RAT RAI (=SIV-50)
SEX : MALE
GROUP : 1 = 0.000 MG/M3

EXP. NO. : R06432/1
COMPOUND : TK 10410

RECOVERY PERIOD

ANIMAL NO.	DAY 24	27	29	31	34	36	38	41	42
1	282	303	288	301	313	325	330	342	334
2	295	315	325	345	354	361	372	389	385
3	258	281	291	304	326	329	339	350	350
4	287	311	316	327	349	352	366	381	376
5	255	275	278	294	304	311	316	328	328

GROUP : 3 = 152.000 MG/M3

RECOVERY PERIOD

ANIMAL NO.	DAY 24	27	29	31	34	36	38	41	42
21	258	279	288	302	315	321	334	347	349
22	256	276	282	301	319	327	331	347	345
23	257	289	295	315	337	345	350	369	367
24	248	273	288	296	310	317	327	341	343
25	256	283	287	310	326	340	347	361	363

INDIVIDUAL BODY WEIGHT

G / ANIMAL

SPECIES : RAT RAI (=SIV-50)
SEX : FEMALE
GROUP : 1 = 0.000 MG/M3

EXP. NO. : R06432/I
COMPOUND : TK 10410

RECOVERY PERIOD

ANIMAL NO.	DAY 24	27	29	31	34	36	38	41	42
41	219	224	229	234	245	247	248	255	255
42	203	212	213	223	218	225	224	232	226
43	202	207	213	216	227	229	235	240	238
44	205	226	228	243	251	246	258	262	264
45	221	226	233	237	250	250	257	266	263

GROUP : 3 = 152.000 MG/M3

RECOVERY PERIOD

ANIMAL NO.	DAY 24	27	29	31	34	36	38	41	42
61	198	213	212	237	245	258	261	262	270
62	203	203	212	212	230	230	236	244	244
63	198	212	209	233	238	245	245	249	250
64	199	215	210	226	229	227	235	241	242
65	196	214	213	229	240	246	246	248	253

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H A L M A T O L Y

EXPERIMENT NO.: SISS-6432

SPECIES : RAT (RAI-OUTBRED)

COMPOUND : TK 10410

DOSE : 0.000 MG/M³

4-WEEK OF TEST

NO. OF ANIMAL	SEX	HB GZ	LKYTHRO-CYTES MILL/CMM	PCV %	ACTICO-LOCYTES %	THROMBO-CYTES 1000 /CMM	LEUCO-CYTES 1000 /CMM	DIFFERENTIAL BLOOD COUNT % BAND	SEG	EO	BA	MO	LY	NEC %/NEC	I.D. %	MCV CU	MCH UUZ	MHB %	P SEC
1.	M	16.6	7.4	51.	5.6	755.	7.0	2.0	11.0	1.0	0.0	0.0	80.0	0.0	0.0	69.	22.	0.5	11.5
2.	M	16.7	7.3	50.	4.6	803.	4.5	0.0	10.0	0.0	0.0	0.0	90.0	0.0	0.0	66.	23.	0.7	11.5
3.	M	15.3	6.8	48.	5.0	708.	6.7	0.0	13.0	0.0	0.0	0.0	87.0	0.0	0.0	71.	23.	0.6	11.0
4.	M	14.8	6.5	46.	5.6	933.	7.5	0.0	20.0	3.0	0.0	0.0	77.0	0.0	0.0	71.	23.	1.0	11.0
5.	M	15.8	7.5	51.	5.2	771.	5.3	0.0	12.0	0.0	0.0	1.0	87.0	0.0	0.0	68.	21.	0.6	11.0
MEAN		15.8	7.1	49.2	5.4	770.0	6.2	0.4	13.2	0.8	0.0	0.2	85.4	0.0	0.0	69.4	22.4	0.7	11.0
41.	F	15.1	7.0	49.	6.0	895.	3.5	1.0	9.0	3.0	0.0	1.0	86.0	0.0	0.0	70.	22.	0.7	10.5
42.	F	16.7	7.0	53.	4.8	985.	4.3	0.0	8.0	1.0	0.0	0.0	91.0	0.0	0.0	70.	22.	0.5	10.0
43.	F	15.3	7.0	49.	5.6	895.	4.6	0.0	15.0	2.0	0.0	1.0	82.0	0.0	0.0	70.	22.	0.7	11.0
44.	F	15.4	7.0	49.	5.2	700.	3.5	0.0	16.0	0.0	0.0	0.0	84.0	0.0	0.0	70.	22.	0.4	11.0
45.	F	14.9	6.8	48.	4.4	1045.	5.2	0.0	12.0	0.0	0.0	0.0	88.0	0.0	0.0	71.	22.	0.9	11.0
MEAN		15.5	7.1	49.6	5.2	907.0	4.1	0.2	12.0	1.4	0.0	0.4	86.2	0.0	0.0	70.2	22.0	0.6	10.9
MEAN M+F		15.7	7.1	49.4	5.3	838.0	5.2	0.3	12.0	1.0	0.0	0.3	85.8	0.0	0.0	69.8	22.2	0.7	11.2

00048

HAEMATOLOGY

EXPERIMENT NO.: SISS-6432

SPECIES : RAT (RAI-OUTBRED)

APPLICATION : INHALATION

COMPOUND: TK 10410

DOSE : 0.000 MG/M³

4. WEEK OF TEST

NO. OF ANIMAL SEX		P T T SEC
1.	M	20.2
2.	M	16.3
3.	M	14.7
4.	M	14.6
5.	M	14.1
MEAN		16.0
41.	F	14.0
42.	F	13.6
43.	F	13.0
44.	F	17.5
45.	F	15.0
MEAN		14.6
MEAN M+F		15.3

00049

HAEMATOLOGY

EXPERIMENT NO.: S155-0000

SPECIES : RAT (RAI-HUTBERG)

COMPOUND : TK 16421

DOSE : 50,000 MG/M².

4-WEEK OF TEST

NO. OF ANIMAL	SEX	HB GR	ERYTHRO-CYTES MILL/CMM	PCV %	RETICULOCYTES %	THROMBOCYTES 1000 /CMM	LEUCOCYTES 1000 /CMM	DIFFERENTIAL BLOOD COUNT % BAND	SEG	EO	BA	MO	LY	ABS. 2/MM6	I.BS. %	MCH PG	MCH GRS	MMG %	P SEC
11.	M	16.4	7.0	49.	0.0	723.	5.0	0.0	15.0	0.0	0.0	0.0	82.0	0.0	0.0	70.	34.	0.5	11.5
12.	M	16.4	6.6	46.	0.0	686.	3.7	2.0	14.0	3.0	0.0	1.0	80.0	0.0	0.0	71.	34.	0.5	12.0
13.	M	16.7	7.0	47.	0.0	737.	6.0	1.0	4.0	1.0	0.0	0.0	94.0	0.0	0.0	68.	34.	0.7	11.0
14.	M	13.0	7.0	49.	0.0	546.	4.0	4.0	8.0	3.0	0.0	0.0	85.0	0.0	0.0	70.	34.	0.0	12.0
15.	M	16.4	7.4	51.	0.0	600.	5.0	2.0	12.0	1.0	0.0	0.0	85.0	0.0	0.0	69.	32.	0.0	11.0
MEAN		16.3	7.2	49.1	0.0	660.4	4.7	1.0	13.4	1.0	0.0	0.0	82.6	0.0	0.0	69.0	33.0	0.0	11.0
51.	F	14.9	6.7	49.	0.0	630.	3.0	2.0	6.0	2.0	0.0	0.0	90.0	0.0	0.0	73.	30.	0.0	11.0
52.	F	14.6	6.6	48.	0.0	750.	3.7	2.0	17.0	1.0	0.0	0.0	79.0	0.0	0.0	72.	31.	0.7	11.3
53.	F	16.1	7.5	51.	0.0	802.	4.2	2.0	8.0	1.0	0.0	0.0	89.0	0.0	0.0	69.	31.	0.0	11.0
54.	F	16.9	7.4	50.	0.0	833.	4.3	1.0	15.0	1.0	0.0	0.0	83.0	0.0	0.0	68.	33.	0.0	11.0
55.	F	16.3	7.0	49.	0.0	1016.	4.3	3.0	5.0	2.0	0.0	0.0	90.0	0.0	0.0	69.	33.	0.0	11.1
MEAN		15.8	7.1	49.4	0.0	699.4	4.0	2.0	11.0	1.4	0.0	0.0	85.6	0.0	0.0	69.4	32.4	0.0	11.4
MEAN M+F		16.1	7.1	49.0	0.0	680.1	4.4	1.2	12.4	1.0	0.0	0.0	83.8	0.0	0.0	69.0	32.4	0.0	11.0

53000

HAEMATOLOGY

EXPERIMENT NO.: SISS-6432

SPECIES : RAT (RAI-OUTBRED)

APPLICATION : INHALATION

COMPOUND: TK 10410

DOSE : 53.000 MG/M³

4. WEEK OF TEST

NO. OF ANIMAL	SEX	P T T SEC
11.	M	14.0
12.	M	16.1
13.	M	19.1
14.	M	18.5
15.	M	12.6
MEAN		16.1
51.	F	15.6
52.	F	14.1
53.	F	14.5
54.	F	12.1
55.	F	14.6
MEAN		14.2
MEAN M+F		15.1

0005

H A E M A T O L Y

EXPERIMENT NO.: 5153-0452 COMPOUND : TK 10410

SPECIES : RAT (RAT-OUTBRED) DOSE : 152.000 MG/M³

4.WEEK OF TEST

NO. OF ANIMAL	SEX	HB G%	ERYTHRO-CYTES MILL/CMM	PCV %	RETICU-LOCYTES %	THROMBO-CYTES 1.00 /CMM	LEUCO-CYTES 1.00 /CMM	DIFFERENTIAL BAND	SEG	EO	BA	MO	LY	BLOOD COUNT % NBL %/NBL	1.00 %	MCV CU.	MCH DUE	MHB %	P SEG
21.	M	13.9	6.3	44.	5.2	500.	5.2	2.0	19.0	0.0	0.0	1.0	78.0	0.0	0.0	70.	22.	0.6	11.1
22.	M	14.3	6.5	46.	5.6	814.	4.1	1.0	21.0	0.0	0.0	0.0	78.0	0.0	0.0	71.	22.	0.5	11.1
23.	M	14.9	6.5	45.	4.0	805.	4.9	0.0	20.0	2.0	0.0	1.0	75.0	0.0	0.0	71.	22.	0.7	10.5
24.	M	14.7	6.8	48.	5.2	861.	4.1	1.0	21.0	0.0	0.0	0.0	88.0	0.0	0.0	71.	22.	0.4	11.1
25.	M	16.6	7.4	51.	1.6	470.	5.8	1.0	15.0	0.0	0.0	0.0	84.0	0.0	0.0	69.	22.	0.4	11.6
MEAN		14.9	6.6	47.0	5.4	705.2	4.8	2.2	16.4	0.4	0.0	0.4	80.6	0.0	0.0	70.4	22.0	0.5	11.1
61.	F	17.1	7.6	52.	5.6	853.	4.2	0.0	19.0	0.0	0.0	0.0	81.0	0.0	0.0	68.	23.	0.4	11.5
62.	F	15.5	7.2	51.	5.2	752.	5.4	1.0	20.0	4.0	0.0	0.0	67.0	0.0	0.0	69.	22.	0.6	12.0
63.	F	15.5	7.1	50.	4.0	1052.	5.9	1.0	19.0	1.0	0.0	0.0	79.0	0.0	0.0	70.	22.	0.5	11.6
64.	F	15.9	7.2	50.	5.2	811.	3.0	3.0	21.0	1.0	0.0	0.0	74.0	1.0	0.0	69.	22.	0.6	11.5
65.	F	16.1	7.1	50.	4.0	811.	4.8	5.0	15.0	1.0	0.0	1.0	78.0	0.0	0.0	70.	23.	0.5	12.0
MEAN		16.0	7.2	50.4	4.0	804.4	4.0	2.0	16.4	1.0	0.0	0.2	77.0	0.2	0.0	69.2	22.4	0.6	11.6
MEAN M+F		15.4	7.0	49.0	5.0	764.8	4.8	2.1	17.4	1.0	0.0	0.3	79.2	0.1	0.0	69.6	22.2	0.5	11.3

HAEMATOLOGY

EXPERIMENT NO.: SISS-6432

SPECIES : RAT (RAI-OUTBRED)

APPLICATION : INHALATION

COMPOUND: TK 10410

DOSE : 152.000 MG/M³

4. WEEK OF TEST

NO. OF ANIMAL	SEX	P T T SEC
21.	M	13.5
22.	M	14.0
23.	M	17.6
24.	M	14.1
25.	M	13.5
MEAN		14.5
61.	F	14.1
62.	F	11.1
63.	F	9.1
64.	F	12.6
65.	F	14.0
MEAN		12.2
MEAN	M+F	13.4

0033

H A E M A T O L O G Y

EXPERIMENT NO.: S155-6432 COMPOUND : TK 16416

SPECIES : RAT (RAI-OUTBREED) DOSE : 365.000 MG/M³

4.WEEK OF TEST

NO. OF ANIMAL	SEX	HB G%	ERYTHRO-CYTES MILL/CMM	PCV %	RETICU-LOCYTES %	THROMBO-CYTES 1000 /CMM	LEUCO-CYTES 1000 /CMM	DIFFERENTIAL BLOOD COUNT % BAND	SEG	LO	EA	MO	LY	NEL %/WBC	I.B. %	MCV CU	MCH UUE	MHB %	P SEC
31.	M	-0.0	-0.0	-0.	-0.0	-0.	-0.0	-0.0	-0.0	-0.0	-0.0	-0.0	-0.0	-0.0	-0.0	-0.	-0.	-0.0	-0.0
32.	M	-0.0	-0.0	-0.	-0.0	-0.	-0.0	-0.0	-0.0	-0.0	-0.0	-0.0	-0.0	-0.0	-0.0	-0.	-0.	-0.0	-0.0
33.	M	-0.0	-0.0	-0.	-0.0	-0.	-0.0	-0.0	-0.0	-0.0	-0.0	-0.0	-0.0	-0.0	-0.0	-0.	-0.	-0.0	-0.0
34.	M	16.4	6.3	0.0	4.8	700.	4.5	2.0	50.0	0.0	0.0	0.0	42.0	0.0	0.0	72.	22.	0.7	11.6
35.	M	-0.0	-0.0	-0.	-0.0	-0.	-0.0	-0.0	-0.0	-0.0	-0.0	-0.0	-0.0	-0.0	-0.0	-0.	-0.	-0.0	-0.0
MEAN		16.4	6.3	60.0	4.8	700.0	4.5	2.0	50.0	0.0	0.0	0.0	42.0	0.0	0.0	72.0	22.0	0.7	11.6
71.	F	-0.0	-0.0	-0.	-0.0	-0.	-0.0	-0.0	-0.0	-0.0	-0.0	-0.0	-0.0	-0.0	-0.0	-0.	-0.	-0.0	-0.0
72.	F	-0.0	-0.0	-0.	-0.0	-0.	-0.0	-0.0	-0.0	-0.0	-0.0	-0.0	-0.0	-0.0	-0.0	-0.	-0.	-0.0	-0.0
73.	F	-0.0	-0.0	-0.	-0.0	-0.	-0.0	-0.0	-0.0	-0.0	-0.0	-0.0	-0.0	-0.0	-0.0	-0.	-0.	-0.0	-0.0
74.	F	-0.0	-0.0	-0.	-0.0	-0.	-0.0	-0.0	-0.0	-0.0	-0.0	-0.0	-0.0	-0.0	-0.0	-0.	-0.	-0.0	-0.0
75.	F	11.6	7.6	57.	5.6	660.	3.7	5.0	50.0	4.0	0.0	0.0	41.0	0.0	0.0	73.	23.	0.6	11.4
MEAN		16.0	7.6	57.0	5.6	660.0	3.7	5.0	50.0	4.0	0.0	0.0	41.0	0.0	0.0	73.0	23.0	0.6	11.4
MEAN M+F		11.2	8.0	56.5	5.2	684.0	4.1	3.5	53.0	2.0	0.0	0.0	41.5	0.0	0.0	72.5	22.5	0.6	11.5

-0.0 = NO OBSERVATION VALUE ENTERED; ANIMAL DIED SPONTANEOUSLY

HAEMATOLOGY

EXPERIMENT NO.: SISS-6432

SPECIES : RAT (RAI-OUTBRED)

APPLICATION : INHALATION

COMPOUND: TK 10410

DOSE : 305.000 MG/M³

4. WEEK OF TEST

NO. OF ANIMAL	SEX	P T T SEC
31.	M	-0.0
32.	M	-0.0
33.	M	-0.0
34.	M	12.0
35.	M	-0.0
MEAN		12.0
71.	F	-0.0
72.	F	-0.0
73.	F	-0.0
74.	F	-0.0
75.	F	11.5
MEAN		11.5
MEAN M+F		11.8

-0.0 = NO OBSERVATION VALUE ENTERED;
ANIMAL DIED SPONTANEOUSLY

B L O O D - C H E M I S T R Y

EXPERIMENT NO.: [illegible]

SPECIES : RAT (RAI-HOLDERED)

COMPOUND : TK AL-10

DOSE : 0.000 MG/M³

4-WEEK OF TEST

NO. OF ANIMAL	SEX	ELECTROLYTES Na MEQ/L	K MEQ/L	CL MEQ/L	CO2 MEQ/L	GLUC MG%	BUN MG%	CHOL MG%	AP KU/L	GOT KU/L	ALBUL G%	PROTEINS G%	ALB %	ELECTROPHORESIS GLOBULINS A1	A2	A3	B	(FIBR.)	G	A/G
4	M	144.	6.4	100.	24.8	82.1	29.	61.	359.	0.	0.	6.2	54.0	15.0	9.4	6.6	23.8	4.0	6.4	1.3
8	F	142.	5.7	99.	25.6	84.3	28.	84.	384.	6.	6.	6.6	54.0	10.3	9.0	8.0	23.1	4.1	6.1	1.2
12	M	143.	6.3	100.	24.4	17.9	23.	95.	320.	0.	6.	6.4	53.0	17.1	5.6	8.6	19.4	4.7	6.8	1.3
16	M	141.	5.6	98.	26.0	19.6	20.	73.	320.	0.	0.	6.5	54.0	17.1	3.0	6.6	18.7	3.4	6.6	1.3
18	M	141.	6.4	98.	23.4	84.9	37.	91.	320.	0.	0.	6.4	52.1	19.2	3.1	6.6	20.3	3.9	6.8	1.4
MEAN		142.6	6.1	99.0	116.4	20.8	25.4	80.0	34.2.2	0.6	6.0	6.4	53.3	17.3	4.0	6.6	20.4	4.0	6.6	1.3
90.	1	142.	5.9	99.	113.	14.8	17.	113.	12.0.	0.	0.	0.5	54.1	17.0	2.8	6.0	17.4	0.0	6.1	1.4
44.	F	142.	5.8	14.1	90.	23.9	24.	14.	44.4.	0.	0.	0.4	53.4	11.0	4.1	0.1	10.0	3.3	1.0	1.4
46.	F	13.0.	5.7	104.	14.4.	20.1	0.1	12.0.	17.4.	6.	0.	0.3	53.1	14.3	4.8	6.6	14.3	3.7	1.7	1.3
49.	F	144.	5.3	100.	110.	11.1.	13.	11.	12.4.	0.	0.	6.7	53.7	14.4	4.8	6.6	13.3	3.3	1.4	1.4
5..	F	144.	5.4	98.	14..	23.1	15.	88.	11.4.	0.	0.	0.7	53.3	13.1	3.3	6.0	11.1	3.4	1.4	1.3
MEAN		14.2.	5.6	99.6	10.4.0	16.6	14.6	9.1.1	21.1.1	0.0	0.0	0.5	53.6	13.6	3.7	6.6	13.6	3.4	1.0	1.4
MEAN MOF		14.3.	5.8	99.4	11.1.1	18.7	15.1	9.1.4	21.1.1	0.0	0.0	0.5	53.7	13.3	3.3	6.6	14.1	3.1	1.4	1.3

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BLOOD - CHEMISTRY

EXPERIMENT NO.: SISS-6432

SPECIES : RAT (RAI-OUTBRED)

APPLICATION : INHALATION

COMPOUND: TK 10410

DOSE : 0.000 MG/M³

4. WEEK OF TEST

NO. OF ANIMAL	SEX	γ - GT MILLI-U./ML	G O T MILLI-U./ML
6.	M	3.1	42.
7.	M	2.8	43.
8.	M	2.9	45.
9.	M	3.7	41.
10.	M	3.6	44.
MEAN		3.2	43.0
46.	F	2.1	40.
47.	F	3.4	40.
48.	F	3.1	42.
49.	F	2.5	39.
50.	F	2.8	38.
MEAN		2.8	39.8
MEAN	M+F	3.0	41.4

0033

B L O O D - C H E M T R Y

EXPERIMENT NO.: SISS-0432 COMPOUND : TR 16410

SPECIES : RAT (RAT-OUTBRED) DOSE : 53.000 MG/M³

4.WEEK OF TEST

NO. OF ANIMAL	SEX	ELECTROLYTES NA (MEQ/L)	K (MEQ/L)	CL (MEQ/L)	GLU-COSE MG%	BUN MG%	GPT (MILLI-U./ML)	LDH (MILLI-U./ML)	AP (MILLI-U./ML)	CHE (KLETT U.)	ACHE (KLETT U.)	PRO-TEINS G%	ALB %	ELECTROPHORESIS GLOBULINS % A1	A2	A3	SB	(FIB.)	G	A/G
16.	M	144.	6.0	99.	117.	20.1	31.	125.	203.	0.	0.	5.4	54.4	17.2	4.6	0.0	21.2	2.6	0.9	1.2
17.	M	149.	5.7	96.	119.	15.8	33.	62.	333.	0.	0.	6.3	53.1	17.3	4.2	0.0	21.3	2.5	0.0	1.2
18.	M	150.	6.3	99.	101.	17.5	32.	100.	310.	0.	0.	6.1	56.1	15.9	3.9	0.0	21.5	2.2	0.5	1.3
19.	M	149.	5.6	100.	127.	19.0	37.	63.	304.	0.	0.	6.2	53.9	17.7	3.7	0.0	22.9	2.2	0.5	1.2
20.	M	144.	5.2	98.	140.	15.9	30.	81.	330.	0.	0.	6.4	53.6	17.7	3.2	0.0	22.0	2.3	0.0	1.2
MEAN		147.2	5.8	98.8	121.4	17.8	32.6	80.2	337.2	0.0	0.0	6.2	54.2	17.2	3.9	0.0	21.9	2.2	0.5	1.2
56.	F	142.	4.3	98.	113.	16.2	30.	53.	230.	0.	0.	6.3	54.5	15.0	3.2	0.0	22.9	3.2	0.8	1.3
57.	F	140.	5.4	104.	113.	17.6	23.	57.	232.	0.	0.	6.4	52.6	16.8	3.3	0.0	24.2	2.2	0.6	1.2
58.	F	141.	5.1	96.	117.	24.2	30.	101.	182.	0.	0.	5.9	55.1	14.7	2.7	0.0	23.3	3.1	1.1	1.3
59.	F	152.	5.7	90.	121.	23.2	26.	97.	19..	0.	0.	6.4	55.5	17.5	2.6	0.0	22.5	2.1	0.0	1.2
60.	F	147.	5.2	100.	132.	22.9	26.	75.	230.	0.	0.	6.3	54.9	13.6	3.4	0.0	23.6	3.1	1.1	1.3
MEAN		147.0	5.1	99.6	116.0	21.2	25.8	82.6	212.6	0.0	0.0	6.3	54.2	15.5	3.1	0.0	23.4	2.9	0.9	1.3
MEAN M+F		147.1	5.5	99.2	119.0	19.5	29.2	84.4	275.2	0.0	0.0	6.2	54.2	16.4	3.5	0.0	22.7	2.5	0.7	1.2

BLOOD - CHEMISTRY

EXPERIMENT NO.: SISS-6432

SPECIES : RAT (RAI-OUTBRED)

APPLICATION : INHALATION

COMPOUND: TK 10410

DOSE : 53.000 MG/M³

4. WEEK OF TEST

NO. OF ANIMAL	SEX	γ - GT MILLI-U./ML	G O T MILLI-U./ML
16.	M	3.5	43.
17.	M	3.1	41.
18.	M	3.6	50.
19.	M	2.1	39.
20.	M	2.8	43.
MEAN		3.0	43.2
56.	F	2.1	43.
57.	F	2.6	45.
58.	F	2.2	50.
59.	F	3.2	41.
60.	F	2.0	43.
MEAN		2.4	44.0
MEAN	M+F	2.7	43.6

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BLOOD CHEMISTRY

EXPERIMENT NO.: 5155-6432

SPECIES : RAT (RAT-LONG-EVANS)

COMPOUND : TA 20415

DOSE : 25,000 MG/M³

AREA OF TEST

NO. OF ANIMALS	SEX	ELECTROLYTES Na MEQ/L	K MEQ/L	CL MEQ/L	GLUCOSE MG%	BUN MG%	CPK	LDH	AP	BIL	ALBUMIN	PROTEINS GM%	A/G RATIO	ELECTROPHORESIS ALBUMIN %	ALPHA 1 %	ALPHA 2 %	BETA %	GAMMA %	A/G
							MICRO-MG/ML			MG%									
20	M	146	5.1	98	103	14	346	78	264	0	0	6.2	52.0	13.3	9.5	9.0	20	4.0	1.0	1.1
21	M	138	5.4	98	103	17	316	116	394	0	0	6.1	52.0	14.4	9.6	9.6	15	4.1	1.1	1.1
40	M	144	5.3	98	107	14	29	91	300	0	0	6.1	53.0	14.0	9.5	8.9	21	3.7	1.0	1.1
49	M	146	5.0	99	93	16	176	96	426	0	0	6.1	54.0	16.5	9.3	8.6	18	3.0	0.9	1.4
50	M	139	5.3	98	93	17	41	78	368	0	0	6.3	50.0	13.4	8.5	8.6	17	2.7	1.1	1.5
MEAN		142	5.2	98	100	16	176	92	369	0.0	0.0	6.2	52.4	13.7	9.5	8.9	18	3.4	1.0	1.3
60	F	140	4.8	98	91	15	196	76	226	0	0	5.9	54.0	15.7	9.4	8.6	22	4.3	1.1	1.3
61	F	140	5.0	100	99	19	236	99	266	0	0	6.4	53.3	14.3	9.2	8.9	21	4.0	1.1	1.3
66	F	138	5.2	101	100	18	186	88	230	0	0	6.0	54.7	16.3	9.0	8.0	20	3.7	0.9	1.4
69	F	139	5.0	98	98	16	146	76	294	0	0	6.4	51.9	16.4	9.4	8.4	21	3.7	0.9	1.4
70	F	140	5.3	98	119	19	23	99	248	0	0	6.3	51.6	14.1	9.6	8.8	23	3.5	1.1	1.4
MEAN		139	5.1	99	101	17	146	88	253	0.0	0.0	6.2	53.3	15.3	9.3	8.6	21	3.9	1.1	1.3
MEAN	MF	140	5.2	98	100	17	161	91	311	0.0	0.0	6.2	52.8	14.5	9.4	8.8	19	3.7	1.1	1.3

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BLOOD - CHEMISTRY

EXPERIMENT NO.: SISS-6432

SPECIES : RAT (RAI-OUTBRED)

APPLICATION : INHALATION

COMPOUND: TK 10410

DOSE : 152.000 MG/M³

4. WEEK OF TEST

NO. OF ANIMAL	SEX	γ - GT MILLI-U./ML	G O T MILLI-U./ML
26.	M	1.9	47.
27.	M	2.0	45.
28.	M	2.2	42.
29.	M	1.7	46.
30.	M	2.9	41.
MEAN		2.1	44.2
66.	F	2.4	36.
67.	F	2.2	39.
68.	F	2.4	39.
69.	F	2.5	40.
70.	F	2.9	36.
MEAN		2.5	38.0
MEAN	M+F	2.3	41.1

0 0 0 1

B L O O D - C H E M T R Y

EXPERIMENT NO.: 515 [illegible]

SPECIES : RAT (RAI-OUTBRED)

COMPOUND : TA [illegible]

DOSE : 305.000 MG/M³

4 WEEK OF TEST

NO. OF ANIMAL	SEX	ELECTROLYTES NA	K	CL	GLU-COSE	BUN	SGT	LDH	AP	CHE	ACHE	PRO-TEINS	ALB	ELECTROPHORESIS GLOBULINS % A1	A2	A3	SB	(FIB.)	G	A/G
		MEQ/L			MG%	MG%	MILLI-U./ML			KELIT U.		G%	%							
36.	M	[illegible]	[illegible]	95.	[illegible]	[illegible]	[illegible]	95.	[illegible]	0.	0.	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
37.	M	[illegible]	[illegible]	95.	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0.	0.	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
38.	M	-0.	-0.0	-0.	-0.	-0.0	-0.	-0.	-0.	0.	0.	-0.0	-0.0	-0.0	-0.0	0.0	-0.0	-0.0	-0.0	-0.0
39.	M	[illegible]	[illegible]	95.	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0.	0.	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
40.	M	-0.	-0.0	-0.	-0.	-0.0	-0.	-0.	-0.	0.	0.	-0.0	-0.0	-0.0	-0.0	0.0	-0.0	-0.0	-0.0	-0.0
MEAN		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0.0	0.0	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
76.	F	-0.	-0.	-0.	-0.	-0.0	-0.	-0.	-0.	0.	0.	-0.0	-0.0	-0.0	-0.0	0.0	-0.0	-0.0	-0.0	-0.0
77.	F	-0.	-0.	-0.	-0.	-0.0	-0.	-0.	-0.	0.	0.	-0.0	-0.0	-0.0	-0.0	0.0	-0.0	-0.0	-0.0	-0.0
78.	F	-0.	-0.	-0.	-0.	-0.0	-0.	-0.	-0.	0.	0.	-0.0	-0.0	-0.0	-0.0	0.0	-0.0	-0.0	-0.0	-0.0
79.	F	-0.	-0.	-0.	-0.	-0.0	-0.	-0.	-0.	0.	0.	-0.0	-0.0	-0.0	-0.0	0.0	-0.0	-0.0	-0.0	-0.0
80.	F	-0.	-0.0	-0.	-0.	-0.0	-0.	-0.	-0.	0.	0.	-0.0	-0.0	-0.0	-0.0	0.0	-0.0	-0.0	-0.0	-0.0
MEAN		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
MEAN M+F		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0.0	0.0	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

-0.0 - NO OBSERVATION ENTERED ; ANIMAL DIED SPONTANEOUSLY

00062

BLOOD - CHEMISTRY

EXPERIMENT NO.: SISS-6432

SPECIES : RAT (RAI-OUTBRED)

APPLICATION : INHALATIOI

COMPOUND: TK 10410

DOSE : 305.000 MG/M³

4. WEEK OF TEST

NO. OF ANIMAL	SEX	γ - GT MILLI-U./ML	G O T MILLI-U./ML
36.	M	2.0	67.
37.	M	2.4	180.
38.	M	-0.0	-0.
39.	M	1.0	52.
40.	M	-0.0	-0.
MEAN		1.8	99.7
76.	F	-0.0	-0.
77.	F	-0.0	-0.
78.	F	-0.0	-0.
79.	F	-0.0	-0.
80.	F	-0.0	-0.
MEAN		0.0	0.0
MEAN	M+F	1.8	99.7

-0.0 = NO OBSERVATION VALUE ENTERED;
ANIMAL DIED SPONTANEOUSLY

MEAN ORGAN WEIGHTS (G) AND RATIOS (AS A PERCENTAGE OF BODY- AND BRAIN WEIGHT)

SPECIES : RAT RAI (=SIV-50)
SEX : MALE

EXP. NO. : R06432/1
COMPOUND : TK 10410

TEST DAY 22

ORGANS	DOSE IN MG/M3 0.000 NO	0.000 MEAN	53.000 NO	53.000 MEAN	152.000 NO	152.000 MEAN	305.000 NO	305.000 MEAN	TREND
+ BODY	5	240.72	10	242.66	5	202.24*	4	136.70*	<--
+ BRAIN	5	1.983	10	1.940	5	1.874	4	1.774*	<--
BRAIN / BODY	5	0.828	10	0.801	5	0.928	4	1.310*	-->
+ HEART	5	0.861	10	0.872	5	0.923	4	0.736	
HEART / BODY	5	0.359	10	0.362	5	0.456*	4	0.535*	-->
HEART / BRAIN	5	43.372	10	45.026	5	49.351	4	41.535	
+ LIVER	5	7.061	10	8.204	5	6.717	4	4.972*	<--
LIVER / BODY	5	2.945	10	3.363	5	3.320	4	3.626	
LIVER / BRAIN	5	356.239	10	422.774	5	359.312	4	280.144*	
+ KIDNEYS	5	1.745	10	1.806	5	1.702	4	1.392*	
KIDNEYS / BODY	5	0.726	10	0.745	5	0.841	4	1.021*	-->
KIDNEYS / BRAIN	5	87.958	10	93.072	5	90.842	4	78.477*	

NO = NO. OF VALUES / GROUP
* = SIGN. DIFFERENCE (LOCATION AND/OR DISPERSION) BETWEEN CONTROL (GROUP 1) AND GROUP X (SIGN.L.= 0.050)
--> = SIGN. POS. TREND FROM CONTROL TO HIGHEST DOSAGE-GROUP (SIGN.L.= 0.010)
<-- = SIGN. NEG. TREND FROM CONTROL TO HIGHEST DOSAGE-GROUP (SIGN.L.= 0.010)

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MEAN ORGAN WEIGHTS (G) AND RATIOS (AS A PERCENTAGE OF BODY- AND BRAIN WEIGHT)

SPECIES : RAT RAI (=SIV-50)
SEX : MALE

EXP. NO. : R06432/1
COMPOUND : TK 10410

TEST DAY 22

ORGANS	DOSE IN MG/M3 0.000 NO	0.000 MEAN	53.000 NO	53.000 MEAN	152.000 NO	152.000 MEAN	305.000 NO	305.000 MEAN	TREND
+ ADRENALS	5	0.048	10	0.046	5	0.050	4	0.052	
ADRENALS / BODY	5	0.020	10	0.019	5	0.025	4	0.038*	-->
ADRENALS / BRAIN	5	2.422	10	2.374	5	2.677	4	2.913	
+ THYMUS	5	0.472	10	0.525	5	0.320	4	0.082*	<--
THYMUS / BODY	5	0.197	10	0.216	5	0.158	4	0.059*	<--
THYMUS / BRAIN	5	23.784	10	27.030	5	17.052	4	4.621*	<--
+ GONADES	5	2.820	10	2.700	5	2.568	4	1.682*	<--
GONADES / BODY	5	1.171	10	1.116	5	1.271	4	1.238	
GONADES / BRAIN	5	142.157	10	139.247	5	137.155	4	95.121	
+ LUNG	5	0.936	10	0.880*	5	0.921	4	0.873	
LUNG / BODY	5	0.389	10	0.363	5	0.455	4	0.652*	-->
LUNG / BRAIN	5	47.183	10	45.435	5	49.300	4	49.231	

NO = NO. OF VALUES / GROUP
* = SIGN. DIFFERENCE (LOCATION AND/OR DISPERSION) BETWEEN CONTROL (GROUP 1) AND GROUP X (SIGN.L.= 0.050)
--> = SIGN. POS. TREND FROM CONTROL TO HIGHEST DOSAGE-GROUP (SIGN.L.= 0.010)
<-- = SIGN. NEG. TREND FROM CONTROL TO HIGHEST DOSAGE-GROUP (SIGN.L.= 0.010)

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MEAN ORGAN WEIGHTS (G) AND RATIOS (AS A PERCENTAGE OF BODY- AND BRAIN WEIGHT)

SPECIES : RAT RAI (=SIV-50)
SEX : FEMALE

EXP. NO. : R06432/1
COMPOUND : TK 10410

TEST DAY 22

ORGANS	DOSE IN MG/M3 0.000		53.000		152.000		305.000		TREND
	NO	MEAN	NO	MEAN	NO	MEAN	NO	MEAN	
+ BODY	5	181.84	10	186.71	5	176.14	1	135.10	
+ BRAIN	5	1.901	10	1.867	5	1.889	1	1.742	
BRAIN / BODY	5	1.047	10	1.001	5	1.073	1	1.289	
+ HEART	5	0.682	10	0.713	5	0.777	1	0.828	
HEART / BODY	5	0.376	10	0.381	5	0.441	1	0.613	
HEART / BRAIN	5	35.856	10	38.102	5	41.048*	1	47.532	
+ LIVER	5	5.552	10	6.365	5	5.522	1	7.165	
LIVER / BODY	5	3.052	10	3.408	5	3.131	1	5.303	
LIVER / BRAIN	5	292.373	10	341.455	5	291.815	1	411.309	
+ KIDNEYS	5	1.410	10	1.428	5	1.470	1	1.374	
KIDNEYS / BODY	5	0.777	10	0.764	5	0.834	1	1.017	
KIDNEYS / BRAIN	5	74.199	10	76.445	5	77.707	1	78.875	

NO = NO. OF VALUES / GROUP
* = SIGN. DIFFERENCE (LOCATION AND/OR DISPERSION) BETWEEN CONTROL (GROUP 1) AND GROUP X (SIGN.L.= 0.05C)
*** NO STATISTICALLY SIGN. TRENDS (SIGN.L.= 0.01C)

00005

MEAN ORGAN WEIGHTS (G) AND RATIOS (AS A PERCENTAGE OF BODY- AND BRAIN WEIGHT)

SPECIES : RAT RAI (=SIV-50) EXP. NO. : R06432/1
SEX : FEMALE COMPOUND : TK 10410

TEST DAY 22

ORGANS	DOSE IN MG/M3 0.000 NO	0.000 MEAN	53.000 NO	53.000 MEAN	152.000 NO	152.000 MEAN	305.000 NO	305.000 MEAN	TREND
+ ADRENALS	5	0.066	10	0.071	5	0.077	1	0.050	
ADRENALS / BODY	5	0.037	10	0.038	5	0.043	1	0.037	
ADRENALS / BRAIN	5	3.489	10	3.603	5	4.054	1	2.870	
+ THYMUS	5	0.430	10	0.372	5	0.363	1	0.109	
THYMUS / BODY	5	0.236	10	0.198	5	0.206	1	0.081	
THYMUS / BRAIN	5	22.704	10	19.913	5	19.176	1	6.257	
+ GONADES	5	0.101	10	0.110	5	0.107	1	0.085	
GONADES / BODY	5	0.056	10	0.059	5	0.061	1	0.063	
GONADES / BRAIN	5	5.304	10	5.881	5	5.663	1	4.879	
+ LUNG	5	0.891	10	0.797*	5	0.853	1	0.764	
LUNG / BODY	5	0.491	10	0.427*	5	0.484	1	0.566	
LUNG / BRAIN	5	46.827	10	42.701	5	45.166	1	43.858	

NO = NO. OF VALUES / GROUP
* = SIGN. DIFFERENCE (LOCATION AND/OR DISPERSION) BETWEEN CONTROL (GROUP 1) AND GROUP X (SIGN.L.= 0.050)
*** NO STATISTICALLY SIGN. TRENDS (SIGN.L.= 0.010)

00097

MEAN ORGAN WEIGHTS (G) AND RATIOS (AS A PERCENTAGE OF BODY- AND BRAIN WEIGHT)

SPECIES : RAT RAI (=SIV-50)
SEX : MALE

EXP. NO. : R06432/1
COMPOUND : TK 10410

TEST DAY 43
RECOVERY PERIOD

ORGANS	DOSE IN MG/M3 0.000 NO	0.000 MEAN	53.000 NO	53.000 MEAN	152.000 NO	152.000 MEAN	305.000 NO	305.000 MEAN	TREND
+ BODY	5	369.90			5	365.60			
+ BRAIN	5	2.012			5	2.047			
BRAIN / BODY	5	0.545			5	0.560			
+ HEART	5	1.129			5	1.168			
HEART / BODY	5	0.305			5	0.319			
HEART / BRAIN	5	55.943			5	57.256			
+ LIVER	5	14.103			5	15.361			
LIVER / BODY	5	3.817			5	4.195			
LIVER / BRAIN	5	700.568			5	752.567			
+ KIDNEYS	5	2.519			5	2.547			
KIDNEYS / BODY	5	0.681			5	0.695			
KIDNEYS / BRAIN	5	124.997			5	124.567			

NO = NO. OF VALUES / GROUP
*** NO STATISTICALLY SIGN. DIFFERENCE BETWEEN CONTROL AND TREATED GROUPS (SIGN.L.= 0.050)
*** NO STATISTICALLY SIGN. TRENDS (SIGN.L.= 0.010)

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MEAN ORGAN WEIGHTS (G) AND RATIOS (AS A PERCENTAGE OF BODY- AND BRAIN WEIGHT)

SPECIES : RAT RAI (=SIV-SC)
SEX : MALE

EXP. NO. : R06432/1
COMPOUND : TK 10410

TEST DAY 43
RECOVERY PERIOD

ORGANS	DOSE IN MG/M3 0.000 NO	0.000 MEAN	53.000 NO	53.000 MEAN	152.000 NO	152.000 MEAN	305.000 NO	305.000 MEAN	TREND
+ ADRENALS	5	0.048			5	0.046			
ADRENALS / BODY	5	0.013			5	0.013			
ADRENALS / BRAIN	5	2.400			5	2.277			
+ THYMUS	5	0.622			5	0.534			
THYMUS / BODY	5	0.167			5	0.146			
THYMUS / BRAIN	5	30.696			5	26.150			
+ GONADES	5	2.951			5	2.949			
GONADES / BODY	5	0.801			5	0.807			
GONADES / BRAIN	5	146.961			5	144.423			
+ LUNG	5	1.149			5	1.216			
LUNG / BODY	5	0.311			5	0.333			
LUNG / BRAIN	5	57.181			5	59.218			

NO = NO. OF VALUES / GROUP
*** NO STATISTICALLY SIGN. DIFFERENCE BETWEEN CONTROL AND TREATED GROUPS (SIGN.L.= 0.050)
*** NO STATISTICALLY SIGN. TRENDS (SIGN.L.= 0.010)

MEAN ORGAN WEIGHTS (G) AND RATIOS (AS A PERCENTAGE OF BODY- AND BRAIN WEIGHT)

SPECIES : RAT RAI (=SIV-50)
SEX : FEMALE

EXP. NO. : R06432/1
COMPOUND : TK 10410

TEST DAY 43
RECOVERY PERIOD

ORGANS	DOSE IN MG/M3 0.000 NO	0.000 MEAN	53.000 NO	53.000 MEAN	152.000 NO	152.000 MEAN	305.000 NO	305.000 MEAN	TREND
+ BODY	5	257.86			5	257.40			
+ BRAIN	5	1.961			5	1.920			
BRAIN / BODY	5	0.760			5	0.749			
+ HEART	5	0.854			5	0.914			
HEART / BODY	5	0.331			5	0.355			
HEART / BRAIN	5	43.806			5	47.756			
+ LIVER	5	10.144			5	10.507			
LIVER / BODY	5	3.951			5	4.080			
LIVER / BRAIN	5	523.323			5	548.971			
+ KIDNEYS	5	1.779			5	1.780			
KIDNEYS / BODY	5	0.691			5	0.692			
KIDNEYS / BRAIN	5	91.252			5	92.921			

NO = NO. OF VALUES / GROUP
*** NO STATISTICALLY SIGN. DIFFERENCE BETWEEN CONTROL AND TREATED GROUPS (SIGN.L.= 0.050)
*** NO STATISTICALLY SIGN. TRENDS (SIGN.L.= 0.010)

MEAN ORGAN WEIGHTS (G) AND RATIOS (AS A PERCENTAGE OF BODY- AND BRAIN WEIGHT)

SPECIES : RAT RAI (=SIV-5C)
SEX : FEMALE

EXP. NO. : R06432/1
COMPOUND : TK 10410

TEST DAY 43
RECOVERY PERIOD

ORGANS	DOSE IN MG/M3 0.000 NO	0.000 MEAN	53.000 NO	53.000 MEAN	152.000 NO	152.000 MEAN	305.000 NO	305.000 MEAN	TREND
+ ADRENALS	5	0.078			5	0.078			
ADRENALS / BODY	5	0.030			5	0.030			
ADRENALS / BRAIN	5	3.966			5	4.043			
+ THYMUS	5	0.518			5	0.468			
THYMUS / BODY	5	0.200			5	0.182			
THYMUS / BRAIN	5	26.382			5	24.394			
+ GONADES	5	0.127			5	0.127			
GONADES / BODY	5	0.049			5	0.049			
GONADES / BRAIN	5	6.533			5	6.622			
+ LUNG	5	0.907			5	1.008			
LUNG / BODY	5	0.351			5	0.393			
LUNG / BRAIN	5	46.173			5	52.516			

ND = NO. OF VALUES / GROUP
*** NO STATISTICALLY SIGN. DIFFERENCE BETWEEN CONTROL AND TREATED GROUPS (SIGN.L.= 0.050)
*** NO STATISTICALLY SIGN. TRENDS (SIGN.L.= 0.010)

INDIVIDUAL ORGAN WEIGHTS (G) AND RATIOS (AS A PERCENTAGE OF BODY- AND BRAIN WEIGHT)

SPECIES : RAT RAI (=SIV-50)
SEX : MALE
GROUP : 1 = 0.000 MG/M3

EXP. NO. : RD6432/1
COMPOUND : TK 10410

TEST DAY 22

ANIMAL NO.	BODY	BRAIN	BRAIN / BODY	HEART	HEART / BODY	HEART / BRAIN	LIVER	LIVER / BODY	LIVER / BRAIN
6	258.40	1.932	0.748	0.889	0.344	46.014	6.960	2.693	360.248
7	230.70	1.975	0.856	0.850	0.368	43.038	7.358	3.189	372.557
8	229.60	1.924	0.838	0.755	0.329	39.241	6.889	3.000	358.056
9	262.00	2.042	0.779	0.885	0.338	43.340	7.249	2.767	354.995
10	222.90	2.043	0.917	0.924	0.415	45.228	6.851	3.074	335.340

GROUP : 2 = 53.000 MG/M3

ANIMAL NO.	BODY	BRAIN	BRAIN / BODY	HEART	HEART / BODY	HEART / BRAIN	LIVER	LIVER / BODY	LIVER / BRAIN
11	249.00	1.942	0.780	0.785	0.315	40.422	9.627	3.866	495.726
12	248.10	1.966	0.792	0.824	0.332	41.913	7.916	3.191	402.645
13	258.90	2.036	0.786	0.928	0.358	45.580	10.293	3.976	505.550
14	257.80	1.936	0.751	0.775	0.301	40.031	9.900	3.840	511.364
15	253.90	1.911	0.753	0.736	0.290	38.514	9.312	3.668	487.284
16	224.90	1.907	0.848	1.069	0.475	56.057	7.805	3.470	409.281
17	229.30	1.772	0.773	0.878	0.383	49.549	6.811	2.970	384.368
18	228.20	2.051	0.899	0.925	0.405	45.100	6.084	2.666	296.636
19	224.70	1.862	0.829	0.915	0.407	49.141	6.458	2.874	346.831
20	251.80	2.018	0.801	0.887	0.352	43.954	7.831	3.110	388.057

INDIVIDUAL ORGAN WEIGHTS (G) AND RATIOS (AS A PERCENTAGE OF BODY- AND BRAIN WEIGHT)

SPECIES : RAT RAI (=SIV-50)
SEX : MALE
GROUP : 1 = 0.000 MG/M3

EXP. NO. : RO6432/1
COMPOUND : TK 10410

TEST DAY 22

ANIMAL NO.	KIDNEYS	KIDNEYS / BODY	KIDNEYS / BRAIN	ADRENALS	ADRENALS / BODY	ADRENALS / BRAIN	THYMUS	THYMUS / BODY	THYMUS / BRAIN
6	1.800	0.697	93.168	0.056	0.022	2.899	0.461	0.178	23.861
7	1.643	0.712	83.190	0.041	0.018	2.076	0.516	0.224	26.127
8	1.600	0.697	83.160	0.045	0.020	2.339	0.372	0.162	19.335
9	1.884	0.719	92.262	0.048	0.018	2.351	0.503	0.192	24.633
10	1.798	0.807	88.008	0.050	0.022	2.447	0.510	0.229	24.963

GROUP : 2 = 53.000 MG/M3

ANIMAL NO.	KIDNEYS	KIDNEYS / BODY	KIDNEYS / BRAIN	ADRENALS	ADRENALS / BODY	ADRENALS / BRAIN	THYMUS	THYMUS / BODY	THYMUS / BRAIN
11	2.067	0.830	106.437	0.047	0.019	2.420	0.710	0.285	36.560
12	1.826	0.736	92.879	0.044	0.018	2.238	0.560	0.226	28.484
13	1.963	0.758	96.415	0.044	0.017	2.161	0.510	0.197	25.049
14	1.917	0.744	99.019	0.054	0.021	2.789	0.564	0.219	29.132
15	1.729	0.681	90.476	0.041	0.016	2.145	0.505	0.199	26.426
16	1.831	0.814	96.015	0.056	0.025	2.937	0.494	0.220	25.905
17	1.510	0.659	85.214	0.046	0.020	2.596	0.402	0.175	22.686
18	1.710	0.749	83.374	0.064	0.028	3.120	0.504	0.221	24.573
19	1.760	0.783	94.522	0.050	0.022	2.685	0.416	0.185	22.342
20	1.743	0.692	86.373	0.013	0.005	0.644	0.588	0.234	29.138

INDIVIDUAL ORGAN WEIGHTS (G) AND RATIOS (AS A PERCENTAGE OF BODY- AND BRAIN WEIGHT)

SPECIES : RAT RAI (=SIV-50)
SEX : MALE
GROUP : 1 = 0.000 MG/M3

EXP. NO. : R06432/1
COMPOUND : TK 10410

TEST DAY 22

ANIMAL NO.	GONADES	GONADES / BODY	GONADES / BRAIN	LUNG	LUNG / BODY	LUNG / BRAIN
6	2.932	1.123	150.207	1.031	0.399	53.364
7	2.742	1.189	138.835	0.984	0.427	49.823
8	2.597	1.131	134.979	0.730	0.318	37.942
9	3.212	1.226	157.297	1.011	0.386	49.510
10	2.645	1.187	129.466	0.925	0.415	45.277

GROUP : 2 = 53.000 MG/M3

ANIMAL NO.	GONADES	GONADES / BODY	GONADES / BRAIN	LUNG	LUNG / BODY	LUNG / BRAIN
11	2.685	1.078	138.259	0.826	0.332	42.533
12	2.646	1.067	134.690	0.857	0.345	43.591
13	2.789	1.077	136.984	0.911	0.352	44.745
14	2.720	1.055	140.496	0.943	0.366	48.709
15	2.708	1.067	141.706	0.897	0.353	46.939
16	2.991	1.330	156.843	0.800	0.356	41.952
17	2.482	1.082	140.068	0.920	0.401	51.919
18	2.838	1.244	138.371	0.920	0.403	44.856
19	2.481	1.104	133.244	0.838	0.373	45.005
20	2.660	1.056	131.814	0.890	0.353	44.103

INDIVIDUAL ORGAN WEIGHTS (G) AND RATIOS (AS A PERCENTAGE OF BODY- AND BRAIN WEIGHT)

SPECIES : RAT RAI (=SIV-50)
SEX : MALE
GROUP : 3 = 152.000 MG/M3

EXP. NO. : R06432/1
COMPOUND : TK 10410

TEST DAY 22

ANIMAL NO.	BODY	BRAIN	BRAIN / BODY	HEART	HEART / BODY	HEART / BRAIN	LIVER	LIVER / BODY	LIVER / BRAIN
26	199.60	1.897	0.950	0.913	0.457	48.129	7.544	3.780	397.680
27	194.30	1.864	0.959	0.829	0.427	44.474	6.226	3.204	334.013
28	215.00	1.850	0.860	1.016	0.473	54.919	7.313	3.401	395.297
29	201.50	1.799	0.893	0.955	0.474	53.085	6.561	3.256	364.702
30	200.80	1.959	0.976	0.924	0.459	46.146	5.943	2.960	303.369

GROUP : 4 = 305.000 MG/M3

ANIMAL NO.	BODY	BRAIN	BRAIN / BODY	HEART	HEART / BODY	HEART / BRAIN	LIVER	LIVER / BODY	LIVER / BRAIN
34	142.50	1.794	1.259	0.781	0.548	43.534	5.326	3.738	296.878
36	134.20	1.784	1.329	0.704	0.525	39.462	5.825	4.341	326.513
37	117.40	1.764	1.503	0.563	0.480	31.916	3.580	3.049	202.948
39	152.70	1.753	1.148	0.898	0.588	51.226	5.158	3.378	294.238

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INDIVIDUAL ORGAN WEIGHTS (G) AND RATIOS (AS A PERCENTAGE OF BODY- AND BRAIN WEIGHT)

SPECIES : RAT RAI (=SIV-50)
SEX : MALE
GROUP : 3 = 152.000 MG/M3

EXP. NO. : R06432/1
COMPOUND : TK 10410

TEST DAY 22

ANIMAL NO.	KIDNEYS	KIDNEYS / BODY	KIDNEYS / BRAIN	ADRENALS	ADRENALS / BODY	ADRENALS / BRAIN	THYMUS	THYMUS / BODY	THYMUS / BRAIN
26	1.821	0.912	95.994	0.059	0.030	3.110	0.276	0.138	14.549
27	1.575	0.811	84.496	0.049	0.025	2.629	0.253	0.130	13.573
28	1.863	0.867	100.703	0.051	0.024	2.757	0.274	0.127	14.811
29	1.545	0.767	85.881	0.042	0.021	2.335	0.384	0.191	21.345
30	1.707	0.850	87.136	0.050	0.025	2.552	0.411	0.205	20.980

GROUP : 4 = 305.000 MG/M3

ANIMAL NO.	KIDNEYS	KIDNEYS / BODY	KIDNEYS / BRAIN	ADRENALS	ADRENALS / BODY	ADRENALS / BRAIN	THYMUS	THYMUS / BODY	THYMUS / BRAIN
34	1.457	1.022	81.215	0.065	0.046	3.623	0.110	0.077	6.132
36	1.463	1.090	82.007	0.055	0.041	3.083	0.067	0.050	3.756
37	1.203	1.022	68.027	0.045	0.038	2.551	0.051	0.043	2.891
39	1.449	0.949	82.658	0.042	0.028	2.396	0.100	0.065	5.705

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INDIVIDUAL ORGAN WEIGHTS (G) AND RATIOS (AS A PERCENTAGE OF BODY- AND BRAIN WEIGHT)

SPECIES : RAT RAI (=SIV-50)
SEX : MALE
GROUP : 3 = 152.000 MG/M3

EXP. NO. : R06432/1
COMPOUND : TK 10410

TEST DAY 22

ANIMAL NO.	GONADES	GONADES / BODY	GONADES / BRAIN	LUNG	LUNG / BODY	LUNG / BRAIN
26	2.586	1.296	136.320	0.969	0.485	51.081
27	2.702	1.391	144.957	0.803	0.413	43.079
28	2.643	1.229	142.865	0.948	0.441	51.243
29	2.422	1.202	134.630	1.062	0.527	59.033
30	2.488	1.239	127.004	0.824	0.410	42.062

GROUP : 4 = 305.000 MG/M3

ANIMAL NO.	GONADES	GONADES / BODY	GONADES / BRAIN	LUNG	LUNG / BODY	LUNG / BRAIN
34	0.810	0.568	45.150	0.770	0.543	42.921
36	1.551	1.156	86.939	0.918	0.684	51.457
37	1.866	1.589	105.782	1.021	0.870	57.880
39	2.500	1.637	142.613	0.783	0.513	44.666

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INDIVIDUAL ORGAN WEIGHTS (G) AND RATIOS (AS A PERCENTAGE OF BODY- AND BRAIN WEIGHT)

SPECIES : RAT RAI (=SIV-50)
SEX : FEMALE
GROUP : 1 = 0.000 MG/M3

EXP. NO. : R06432/1
COMPOUND : TK 10410

TEST DAY 22

ANIMAL NO.	BODY	BRAIN	BRAIN / BODY	HEART	HEART / BODY	HEART / BRAIN	LIVER	LIVER / BODY	LIVER / BRAIN
46	190.30	1.872	0.984	0.640	0.336	34.188	6.148	3.231	328.419
47	187.80	1.902	1.013	0.686	0.365	36.067	5.382	2.866	282.965
48	169.80	1.827	1.076	0.667	0.393	36.508	5.390	3.174	295.019
49	174.70	1.947	1.114	0.733	0.420	37.648	4.940	2.828	253.724
50	186.60	1.956	1.048	0.682	0.365	34.867	5.902	3.163	301.738

GROUP : 2 = 53.000 MG/M3

ANIMAL NO.	BODY	BRAIN	BRAIN / BODY	HEART	HEART / BODY	HEART / BRAIN	LIVER	LIVER / BODY	LIVER / BRAIN
51	180.70	1.800	0.996	0.617	0.341	34.278	6.996	3.872	388.667
52	184.10	1.695	0.921	0.561	0.305	33.097	6.534	3.549	385.487
53	178.70	1.849	1.035	0.570	0.319	30.827	6.587	3.686	356.247
54	200.90	1.911	0.951	0.837	0.417	43.799	7.292	3.630	381.580
55	188.60	1.914	1.015	0.644	0.341	33.647	8.474	4.493	442.738
56	186.70	1.892	1.013	0.807	0.432	42.653	5.385	2.884	284.619
57	191.80	1.923	1.003	0.812	0.423	42.226	5.759	3.003	299.480
58	175.50	1.815	1.034	0.757	0.431	41.708	4.968	2.831	273.719
59	189.60	1.907	1.006	0.826	0.436	43.314	6.021	3.176	315.731
60	190.50	1.968	1.033	0.698	0.366	35.467	5.634	2.957	286.280

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INDIVIDUAL ORGAN WEIGHTS (G) AND RATIOS (AS A PERCENTAGE OF BODY- AND BRAIN WEIGHT)

SPECIES : RAT RAI (=SIV-50) EXP. NO. : R06432/1
SEX : FEMALE COMPOUND : TK 10410
GROUP : 1 = 0.000 MG/M3

TEST DAY 22

ANIMAL NO.	KIDNEYS	KIDNEYS / BODY	KIDNEYS / BRAIN	ADRENALS	ADRENALS / BODY	ADRENALS / BRAIN	THYMUS	THYMUS / BODY	THYMUS / BRAIN
46	1.436	0.755	76.709	0.058	0.030	3.098	0.552	0.290	29.487
47	1.347	0.717	70.820	0.065	0.035	3.417	0.410	0.218	21.556
48	1.409	0.830	77.121	0.062	0.037	3.394	0.446	0.263	24.412
49	1.406	0.805	72.214	0.077	0.044	3.955	0.345	0.197	17.720
50	1.450	0.777	74.131	0.070	0.038	3.579	0.398	0.213	20.348

GROUP : 2 = 53.000 MG/M3

ANIMAL NO.	KIDNEYS	KIDNEYS / BODY	KIDNEYS / BRAIN	ADRENALS	ADRENALS / BODY	ADRENALS / BRAIN	THYMUS	THYMUS / BODY	THYMUS / BRAIN
51	1.268	0.702	70.444	0.048	0.027	2.667	0.323	0.179	17.944
52	1.347	0.732	79.469	0.051	0.028	3.009	0.442	0.240	26.077
53	1.441	0.806	77.934	0.068	0.038	3.678	0.299	0.167	16.171
54	1.524	0.759	79.749	0.064	0.032	3.349	0.387	0.193	20.251
55	1.350	0.716	70.533	0.080	0.042	4.180	0.410	0.217	21.421
56	1.445	0.774	76.374	0.082	0.044	4.334	0.386	0.207	20.402
57	1.575	0.821	81.903	0.056	0.029	2.912	0.440	0.229	22.881
58	1.351	0.770	74.435	0.087	0.050	4.793	0.200	0.114	11.019
59	1.471	0.776	77.137	0.073	0.039	3.828	0.389	0.205	20.399
60	1.505	0.790	76.474	0.104	0.055	5.285	0.444	0.233	22.561

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INDIVIDUAL ORGAN WEIGHTS (G) AND RATIOS (AS A PERCENTAGE OF BODY- AND BRAIN WEIGHT)

SPECIES : RAT RAI (=SIV-50)
SEX : FEMALE
GROUP : 1 = 0.000 MG/M3

EXP. NO. : R06432/1
COMPOUND : TK 10410

TEST DAY 22

ANIMAL NO.	GONADES	GONADES / BODY	GONADES / BRAIN	LUNG	LUNG / BODY	LUNG / BRAIN
46	0.093	0.049	4.968	0.910	0.478	48.611
47	0.095	0.051	4.995	0.870	0.463	45.741
48	0.104	0.061	5.692	0.768	0.452	42.036
49	0.104	0.060	5.342	1.056	0.604	54.237
50	0.108	0.058	5.521	0.851	0.456	43.507

GROUP : 2 = 53.000 MG/M3

ANIMAL NO.	GONADES	GONADES / BODY	GONADES / BRAIN	LUNG	LUNG / BODY	LUNG / BRAIN
51	0.098	0.054	5.444	0.728	0.403	40.444
52	0.112	0.061	6.608	0.783	0.425	46.195
53	0.093	0.052	5.030	0.727	0.407	39.319
54	0.124	0.062	6.489	0.858	0.427	44.898
55	0.109	0.058	5.695	0.785	0.416	41.014
56	0.094	0.050	4.968	0.822	0.440	43.446
57	0.141	0.074	7.332	0.799	0.417	41.550
58	0.098	0.056	5.399	0.802	0.457	44.187
59	0.127	0.067	6.660	0.836	0.441	43.838
60	0.102	0.054	5.183	0.829	0.435	42.124

00080

INDIVIDUAL ORGAN WEIGHTS (G) AND RATIOS (AS A PERCENTAGE OF BODY- AND BRAIN WEIGHT)

SPECIES : RAT RAI (=SIV-50) EXP. NO. : R06432/1
SEX : FEMALE COMPOUND : TK 10410
GROUP : 3 = 152.000 MG/M3

TEST DAY 22

ANIMAL NO.	BODY	BRAIN	BRAIN / BODY	HEART	HEART / BODY	HEART / BRAIN	LIVER	LIVER / BODY	LIVER / BRAIN
66	186.40	1.914	1.027	0.743	0.399	38.819	5.681	3.048	296.813
67	167.50	1.815	1.084	0.684	0.408	37.686	5.042	3.010	277.796
68	184.40	2.040	1.106	0.882	0.478	43.235	6.351	3.444	311.323
69	172.80	1.885	1.091	0.865	0.501	45.889	5.451	3.155	289.178
70	169.60	1.790	1.055	0.739	0.418	39.609	5.083	2.997	283.966

GROUP : 4 = 305.000 MG/M3

ANIMAL NO.	BODY	BRAIN	BRAIN / BODY	HEART	HEART / BODY	HEART / BRAIN	LIVER	LIVER / BODY	LIVER / BRAIN
75	135.10	1.742	1.289	0.828	0.613	47.532	7.165	5.303	411.309

00063

INDIVIDUAL ORGAN WEIGHTS (G) AND RATIOS (AS A PERCENTAGE OF BODY- AND BRAIN WEIGHT)

SPECIES : RAT RAI (=SIV-50)
SEX : FEMALE
GROUP : 3 = 152.000 MG/M3

EXP. NO. : R06432/1
COMPOUND : TK 10410

TEST DAY 22

ANIMAL NO.	KIDNEYS	KIDNEYS / BODY	KIDNEYS / BRAIN	ADRENALS	ADRENALS / BODY	ADRENALS / BRAIN	THYMUS	THYMUS / BODY	THYMUS / BRAIN
66	1.482	0.795	77.429	0.088	0.047	4.598	0.350	0.188	18.286
67	1.349	0.805	74.325	0.068	0.041	3.747	0.363	0.217	20.000
68	1.673	0.906	81.863	0.099	0.054	4.853	0.400	0.217	19.608
69	1.507	0.872	79.947	0.068	0.039	3.607	0.438	0.253	23.236
73	1.342	0.791	74.972	0.062	0.037	3.464	0.264	0.156	14.749

GROUP : 4 = 305.000 MG/M3

ANIMAL NO.	KIDNEYS	KIDNEYS / BODY	KIDNEYS / BRAIN	ADRENALS	ADRENALS / BODY	ADRENALS / BRAIN	THYMUS	THYMUS / BODY	THYMUS / BRAIN
75	1.374	1.017	78.875	0.050	0.037	2.870	0.109	0.081	6.257

INDIVIDUAL ORGAN WEIGHTS (G) AND RATIOS (AS A PERCENTAGE OF BODY- AND BRAIN WEIGHT)

SPECIES : RAT RAI (=SIV-50)
SEX : FEMALE
GROUP : 3 = 152.000 MG/M3

EXP. NO. : R06432/1
COMPOUND : TK 10410

TEST DAY 22

ANIMAL NO.	GONADES	GONADES / BODY	GONADES / BRAIN	LUNG	LUNG / BODY	LUNG / BRAIN
66	0.136	0.057	5.538	0.876	0.470	45.768
67	0.137	0.064	5.895	0.782	0.467	43.085
68	0.125	0.068	6.127	0.931	0.505	45.637
69	0.110	0.064	5.836	0.795	0.460	42.175
70	0.088	0.052	4.916	0.880	0.519	49.162

GROUP : 4 = 305.000 MG/M3

ANIMAL NO.	GONADES	GONADES / BODY	GONADES / BRAIN	LUNG	LUNG / BODY	LUNG / BRAIN
75	0.085	0.063	4.879	0.764	0.566	43.858

INDIVIDUAL ORGAN WEIGHTS (G) AND RATIOS (AS A PERCENTAGE OF BODY- AND BRAIN WEIGHT)

SPECIES : RAT RAI (=SIV-50)
SEX : MALE
GROUP : 1 = 0.000 MG/M3

EXP. NO. : R06432/1
COMPOUND : TK 10410

TEST DAY 43
RECOVERY PERIOD

ANIMAL NO.	BODY	BRAIN	BRAIN / BODY	HEART	HEART / BODY	HEART / BRAIN	LIVER	LIVER / BODY	LIVER / BRAIN
1	347.60	2.001	0.576	1.199	0.345	59.920	13.997	4.027	699.500
2	402.00	2.114	0.526	1.158	0.288	54.778	15.004	3.732	709.745
3	368.60	1.984	0.538	1.056	0.286	53.226	13.501	3.663	680.494
4	393.90	2.106	0.535	1.307	0.332	62.061	15.061	3.824	715.147
5	337.40	1.856	0.550	0.923	0.274	49.731	12.954	3.839	697.952

GROUP : 3 = 152.000 MG/M3

RECOVERY PERIOD

ANIMAL NO.	BODY	BRAIN	BRAIN / BODY	HEART	HEART / BODY	HEART / BRAIN	LIVER	LIVER / BODY	LIVER / BRAIN
21	358.40	2.208	0.616	1.029	0.287	46.603	14.423	4.024	653.216
22	358.00	2.051	0.573	1.165	0.325	56.802	14.112	3.942	688.055
23	381.40	1.952	0.512	1.171	0.307	59.990	16.723	4.385	856.711
24	354.70	1.919	0.541	1.126	0.317	58.676	14.268	4.023	743.512
25	375.50	2.104	0.560	1.351	0.360	64.211	17.281	4.602	821.340

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INDIVIDUAL ORGAN WEIGHTS (G) AND RATIOS (AS A PERCENTAGE OF BODY- AND BRAIN WEIGHT)

SPECIES : RAT RAI (=SIV-50)
SEX : MALE
GROUP : 1 = 0.000 MG/M3

EXP. NO. : R06432/1
COMPOUND : TK 10410

TEST DAY 43
RECOVERY PERIOD

ANIMAL NO.	KIDNEYS	KIDNEYS / BODY	KIDNEYS / BRAIN	ADRENALS	ADRENALS / BODY	ADRENALS / BRAIN	THYMUS	THYMUS / BODY	THYMUS / BRAIN
1	2.643	0.760	132.084	0.041	0.012	2.049	0.591	0.170	29.535
2	2.956	0.735	139.830	0.061	0.015	2.886	0.810	0.201	38.316
3	2.276	0.617	114.718	0.036	0.010	1.815	0.664	0.180	33.468
4	2.495	0.633	118.471	0.055	0.014	2.612	0.648	0.165	30.769
5	2.225	0.659	119.881	0.049	0.015	2.640	0.397	0.118	21.390

GROUP : 3 = 152.000 MG/M3

RECOVERY PERIOD

ANIMAL NO.	KIDNEYS	KIDNEYS / BODY	KIDNEYS / BRAIN	ADRENALS	ADRENALS / BODY	ADRENALS / BRAIN	THYMUS	THYMUS / BODY	THYMUS / BRAIN
21	2.288	0.638	103.623	0.040	0.011	1.812	0.559	0.156	25.317
22	2.477	0.692	120.770	0.052	0.015	2.535	0.484	0.135	23.598
23	2.576	0.675	131.967	0.048	0.013	2.459	0.674	0.177	34.529
24	2.206	0.622	114.956	0.045	0.013	2.345	0.429	0.121	22.355
25	3.188	0.849	151.521	0.047	0.013	2.234	0.525	0.140	24.952

INDIVIDUAL ORGAN WEIGHTS (G) AND RATIOS (AS A PERCENTAGE OF BODY- AND BRAIN WEIGHT)

SPECIES : RAT RAI (=SIV-50)
SEX : MALE
GROUP : 1 = 0.000 MG/M3

EXP. NO. : R06432/1
COMPOUND : TK 10410

TEST DAY 43
RECOVERY PERIOD

ANIMAL NO.	GONADES	GONADES / BODY	GONADES / BRAIN	LUNG	LUNG / BODY	LUNG / BRAIN
1	2.905	0.836	145.177	1.045	0.301	52.224
2	3.188	0.793	150.804	1.266	0.315	59.886
3	2.926	0.794	147.480	1.186	0.322	59.778
4	2.752	0.699	130.674	1.119	0.284	53.134
5	2.982	0.884	160.668	1.130	0.335	60.884

GROUP : 3 = 152.000 MG/M3

RECOVERY PERIOD

ANIMAL NO.	GONADES	GONADES / BODY	GONADES / BRAIN	LUNG	LUNG / BODY	LUNG / BRAIN
21	3.363	0.938	152.310	1.467	0.409	66.440
22	2.629	0.734	128.181	1.275	0.356	62.165
23	3.292	0.863	168.648	1.108	0.291	56.762
24	2.909	0.820	151.589	1.023	0.288	53.309
25	2.554	0.680	121.368	1.208	0.322	57.414

INDIVIDUAL ORGAN WEIGHTS (G) AND RATIOS (AS A PERCENTAGE OF BODY- AND BRAIN WEIGHT)

SPECIES : RAT RAI (=SIV-50)
SEX : FEMALE
GROUP : 1 = 0.000 MG/M3

EXP. NO. : R06432/1
COMPOUND : TK 10410

TEST DAY 43
RECOVERY PERIOD

ANIMAL NO.	BODY	BRAIN	BRAIN / BODY	HEART	HEART / BODY	HEART / BRAIN	LIVER	LIVER / BODY	LIVER / BRAIN
41	256.80	1.922	0.748	0.858	0.334	44.641	9.250	3.602	481.269
42	245.40	1.994	0.813	0.754	0.307	37.813	9.849	4.013	493.932
43	247.20	1.741	0.704	0.909	0.368	52.211	12.589	5.093	723.090
44	274.60	2.065	0.752	0.853	0.311	41.307	9.846	3.586	476.804
45	265.30	2.081	0.784	0.896	0.338	43.056	9.188	3.463	441.518

GROUP : 3 = 152.000 MG/M3

RECOVERY PERIOD

ANIMAL NO.	BODY	BRAIN	BRAIN / BODY	HEART	HEART / BODY	HEART / BRAIN	LIVER	LIVER / BODY	LIVER / BRAIN
61	276.50	1.780	0.644	0.984	0.356	55.281	11.396	4.122	640.225
62	239.20	1.967	0.822	0.902	0.377	45.857	9.361	3.913	475.902
63	261.10	1.983	0.759	0.882	0.338	44.478	10.280	3.937	518.406
64	248.10	1.932	0.779	0.830	0.335	42.961	10.453	4.213	541.046
65	262.10	1.940	0.740	0.974	0.372	50.206	11.044	4.214	569.278

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INDIVIDUAL ORGAN WEIGHTS (G) AND RATIOS (AS A PERCENTAGE OF BODY- AND BRAIN WEIGHT)

SPECIES : RAT RAI (=SIV-50)
SEX : FEMALE
GROUP : 1 = 0.000 MG/M3

EXP. NO. : R06432/1
COMPOUND : TK 10410

TEST DAY 43
RECOVERY PERIOD

ANIMAL NO.	KIDNEYS	KIDNEYS / BODY	KIDNEYS / BRAIN	ADRENALS	ADRENALS / BODY	ADRENALS / BRAIN	THYMUS	THYMUS / BODY	THYMUS / BRAIN
41	1.730	0.674	90.010	0.069	0.027	3.590	0.443	0.173	23.049
42	1.701	0.693	85.306	0.078	0.032	3.912	0.426	0.174	21.364
43	1.875	0.758	107.697	0.071	0.029	4.078	0.497	0.201	28.547
44	1.836	0.669	88.910	0.089	0.032	4.310	0.480	0.175	23.245
45	1.755	0.662	84.334	0.062	0.031	3.940	0.743	0.280	35.704

GROUP : 3 = 152.000 MG/M3

RECOVERY PERIOD

ANIMAL NO.	KIDNEYS	KIDNEYS / BODY	KIDNEYS / BRAIN	ADRENALS	ADRENALS / BODY	ADRENALS / BRAIN	THYMUS	THYMUS / BODY	THYMUS / BRAIN
61	1.841	0.666	103.427	0.075	0.027	4.213	0.434	0.157	24.382
62	1.745	0.730	88.714	0.076	0.032	3.864	0.413	0.173	20.996
63	1.713	0.656	86.384	0.083	0.032	4.186	0.510	0.195	25.719
64	1.673	0.674	86.594	0.069	0.028	3.571	0.462	0.186	23.913
65	1.930	0.736	99.485	0.085	0.032	4.381	0.523	0.200	26.959

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INDIVIDUAL ORGAN WEIGHTS (G) AND RATIOS (AS A PERCENTAGE OF BODY- AND BRAIN WEIGHT)

SPECIES : RAT RAI (=SIV-50)
SEX : FEMALE
GROUP : 1 = 0.000 MG/M3

EXP. NO. : R06432/1
COMPOUND : TK 10410

TEST DAY 43
RECOVERY PERIOD

ANIMAL NO.	GONADES	GONADES / BODY	GONADES / BRAIN	LUNG	LUNG / BODY	LUNG / BRAIN
41	0.130	0.051	6.764	0.912	0.355	47.451
42	0.118	0.048	5.918	0.922	0.376	46.239
43	0.143	0.058	8.214	0.757	0.306	43.481
44	0.118	0.043	5.714	1.011	0.368	48.959
45	0.126	0.047	6.055	0.931	0.351	44.738

GROUP : 3 = 152.000 MG/M3

RECOVERY PERIOD

ANIMAL NO.	GONADES	GONADES / BODY	GONADES / BRAIN	LUNG	LUNG / BODY	LUNG / BRAIN
61	0.135	0.049	7.584	0.944	0.341	53.034
62	0.116	0.048	5.897	0.985	0.412	50.076
63	0.126	0.048	6.354	0.918	0.352	46.293
64	0.122	0.049	6.315	1.126	0.454	58.282
65	0.135	0.052	6.959	1.065	0.406	54.897

Compound: TK 10 410
Experiment No.: Siss. 6432
Hist. No.: Ex 495

Pathology Report

SUMMARY

Six out of 10 male rats and 9 out of 10 female animals from the highest concentration group (305 mg/m3) died spontaneously during the test period. In all these animals acute congestion, and occasional haemorrhages in the myocardium, lungs, liver, kidneys, adrenals, pituitary, ovaries and brain were found.

Furthermore, all rats of this group displayed marked congestion of the nasal mucosa with purulent inflammation and ulceration. In 3 of these rats ulceration of the skin of the muzzle was also noted. All animals from this group showed depletion of thymocytes in the thymus, 5 out of the 10 male rats showed reduced spermatogenesis and in 6 animals atrophy of the lymphoid tissue of the spleen was observed.

Less pronounced congestion and purulent inflammation with ulceration of the nasal mucosa were seen in the muzzle in 6 out of 10 rats from the intermediate concentration group (152 mg/m3). The nasal changes proved to be reversible after a recovery period of 21 days to which one group of animals exposed to the same concentration level (152 mg/m3) was subjected. Of this recovery group only one out of 10 rats showed in the muzzle remnants of a partially re-epithelized ulceration, foreign body reaction and chronic inflammatory infiltration at the base. One male rat only from this group showed arrest of spermatogenesis in one testicle. The experimental significance of this finding is doubtful since unilaterally inhibited spermatogenesis is known to occur spontaneously in rats. All other pathological findings seen in the treated and control animals were considered incidental in nature and not related to the inhalation of TK 10 410.

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Section Head Pathology

V. Skorpil. M.D., Ph.D.
Pathologist

Compound TK 1041o

Methods

At the end of the 21 day toxicity study or after an additional recovery period of 21 days all treated and control rats, which survived the experiment, were bled under ether anaesthesia and complete autopsy was carried out. The total weight of each animal was determined and the following organs were weighed: heart, lungs, liver, kidneys, adrenals, thymus, brain and gonads.

Microscopical Examination

Tissue portions of liver, spleen, kidneys, stomach, small and large intestine, lungs, gonads, prostate or uterus, adrenals, cervical and abdominal lymph nodes, trachea, thyroid, heart, aorta, striated muscle, skin, mammary gland, pancreas, thymus, urinary bladder, pituitary, muzzle, brain and eye ball with optic nerve were fixed in buffered 10 % neutral formalin. The fixed tissue samples from both control and treated animals were embedded in paraffin wax and cut at 3-5 µ. The routine stain was haematoxylin and eosin. Sections from liver, spleen, lungs and kidneys were stained for iron by the Perl method. Sections from brain were stained with Luxol-fast blue / cresylviolet. Additional frozen sections from liver were specifically stained for fat with Sudan III. Complete autopsy and histological examination were performed also in the animals which died during the experiment.

Compound: TK 10'410

Results

Macroscopical Findings

All details regarding the gross anatomical changes are listed among the macroscopical findings of individual rats. All animals from the highest concentration group were smaller than the control rats. No other gross anatomical changes were noted in the treated and control rats, that were considered of experimental relevance.

Microscopical Findings

All details regarding the histopathological changes are listed among the microscopical findings of individual rats.

In all rats from the highest concentration group (305 mg/m^3) marked congestion and purulent inflammation with ulceration of the nasal mucosa were observed. In 3 of these rats ulceration in the skin of the muzzle was also seen. All animals from this group showed depletion of the thymocytes in the thymus, six rats had shrunken spleen with atrophy of the lymphoid tissue. Reduced spermatogenesis was found in 5 out of 10 male rats from this group.

In all 15 animals from the highest concentration group, which died during the experiment period, acute congestion sometimes with haemorrhages in the myocardium, lungs, liver, kidneys, adrenals, pituitary, ovaries and occasional in the brain were found. Slight or minimal congestion and purulent inflammation with ulceration of the nasal mucosa were seen in 6 out of 10 rats from the intermediate concentration group (152 mg/m^3). One rat (16 m) from the lowest dosage group which died on the 23rd exp. day after the termination of the experiment period following blood collection had slight congestion in the heart, spleen, liver, adrenals, kidneys and pituitary.

After a recovery period of 21 days to which one group of animals exposed to the intermediate concentration level was subjected, only one rat (23 m) showed in the muzzle in one place remnant of partially re-epithelized ulcus with a foreign body granuloma and chronic inflammatory infiltration in the base. In one male rat (25) complete arrest of spermatogenesis in one testicle was seen.

All other microscopical findings seen in the treated and control animals and listed among the microscopical findings of individual rats were only incidental in nature and not related to the inhalation of TK 10'410.

Organ Weights

Apart from intergroup changes (also related to the lower body weights of the animals of group 4) there were no obvious differences in the organ weights, organ to body weight and organ to brain weight ratios between the treated and control rats.

Compound: TK 10 410
Hist.No.: Ex 495

Macroscopical and Microscopical Findings in Individual Rats

In order to avoid needless repetition only the pathological changes are reported.

Group 1 - controls - 0 mg/m3

6 m	Macro:	Right eye with traumatic haemorrhages.
	Micro:	Spleen: minimal focal haemosiderosis.
		Liver: slight irregular fatty changes of the hepatocytes.
		Right eye: no changes seen.
7 m	Macro:	Right eye with traumatic haemorrhages.
	Micro:	Liver: slight irregular fatty changes of the hepatocytes.
		Bladder: dilated, with eosinophilic precipitates within the lumen.
		Right eye: minimal peribulbar haemorrhages.
8 m	Macro:	Right eye with traumatic haemorrhage.
	Micro:	Liver: minimal irregular fatty changes of the hepatocytes.
		Right eye: minute peribulbar haemorrhage.
9 m	Macro:	Right eye with traumatic haemorrhage.
	Micro:	Liver: slight irregular fatty changes of the hepatocytes.
		Right eye: minute peribulbar haemorrhage.
10 m	Macro:	Right eye with traumatic haemorrhages.
	Micro:	Liver: slight irregular fatty changes of the hepatocytes.
		Right eye: minimal peribulbar haemorrhage.
46 f	Macro:	Right eye with traumatic haemorrhage.
	Micro:	Spleen: minute extramedullary haematopoiesis and focal haemosiderosis.
		Liver: slight irregular fatty changes of the hepatocytes.
		Right eye: minute peribulbar haemorrhage.
47 f	Macro:	Right eye with traumatic haemorrhage.
	Micro:	Spleen: minute focal haemosiderosis.
		Right eye: slight peribulbar haemorrhage.
		Pituitary not sent for histopathology.
48 f	Macro:	Right eye with traumatic haemorrhage.
	Micro:	Spleen: slight focal haemosiderosis.
		Liver: minute irregular fatty changes of the hepatocytes.
		Kidneys: minimal nephrocalcinosis.
		Right eye: minute peribulbar haemorrhage.
49 f	Macro:	Right eye with traumatic haemorrhage, lungs inflated.
	Micro:	Lungs: slight focal collapse.

Compound: TK 10 410

Spleen: slight focal haemosiderosis
Liver: slight irregular fatty changes of the hepatocytes.
Kidneys: minimal nephrocalcinosis.
Right eye: minute peribulbar haemorrhage.

50 f Macro: Right eye with traumatic haemorrhage.
Lungs: slight inflated.
Micro: Lungs: minimal focal collapse.
Spleen: slight focal haemosiderosis.
Liver: slight irregular fatty changes of the hepatocytes.
Right eye: no changes seen.

Group 2 - 53 mg/m3

11 m Macro: Right eye with traumatic haemorrhage.
Micro: Spleen: minute focal haemosiderosis.
Liver: minimal irregular fatty changes of the hepatocytes.
Right eye: minute peribulbar posttraumatic haemorrhage.

12 m Macro: Right eye with traumatic haemorrhage.
Lungs: inflated.
Micro: Lungs: slight focal collapse.
Urinary bladder: dilated, with eosinophilic precipitates within the lumen.
Right eye: minute peribulbar traumatic haemorrhage.

13 m Macro: Lungs with few nodules.
Micro: Lungs: small areas of collapse.

14 m Macro: No changes seen.
Micro: Urinary bladder: dilated, with eosinophilic precipitates within the lumen.

15 m Macro: Right eye with traumatic haemorrhage.
Micro: Urinary bladder: dilated, with eosinophilic precipitates within the lumen.
Right eye: minute peribulbar traumatic haemorrhage.
Muzzle: slight oedema and congestion of the mucous membrane.

16 m - died after blood collection after the termination of the experiment.
Macro: Both eyes with small traumatic haemorrhages.
Lungs: with some nodules
Micro: Lungs: acute congestion, few lymphohistiocytic infiltrates in the interstitium.
Heart: congestion.

Compound: TK 10 410

		Spleen: slight congestion.
		Liver: slight irregular fatty changes of the hepatocytes, slight congestion.
		Adrenals: congestion.
		Kidneys: slight congestion.
		Pituitary: slight congestion.
		Both eyes: minute traumatic peribulbar haemorrhages.
17 m	Macro:	No changes seen.
	Micro:	Liver: slight irregular fatty changes of the hepatocytes.
		Urinary bladder: dilated, with eosinophilic precipitates within the lumen.
18 m	Macro:	Right eye with traumatic haemorrhage.
		Lungs with some nodules.
	Micro:	Lungs: small areas of collapse.
		Spleen: minute focal haemosiderosis.
		Liver: slight irregular fatty changes of the hepatocytes.
		Right eye: minimal peribulbar haemorrhage.
19 m	Macro:	Right eye with traumatic haemorrhages.
	Micro:	Right eye: minimal peribulbar haemorrhage.
20 m	Macro:	Right eye with slight traumatic haemorrhage.
	Micro:	Liver: slight irregular fatty changes of the hepatocytes.
		Right eye: minimal peribulbar haemorrhage.
51 f	Macro:	Right eye with small traumatic haemorrhage.
	Micro:	Spleen: minute focal haemosiderosis.
		Kidneys: minute nephrocalcinosis.
		Right eye: no changes seen.
52 f	Macro:	Right eye with traumatic haemorrhages.
	Micro:	Spleen: minute focal haemosiderosis.
		Right eye: minimal peribulbar haemorrhage.
53 f	Macro:	Eyes with traumatic haemorrhages.
		Lungs mottled.
	Micro:	Lungs: no changes seen.
		Spleen: minute focal haemosiderosis.
		Kidneys: minimal nephrocalcinosis.
		Eyes: minimal peribulbar haemorrhage.
54 f	Macro:	Right eye with traumatic haemorrhage.
	Micro:	Spleen: slight focal haemosiderosis.
		Kidneys: slight nephrocalcinosis.
		Right eye: minimal peribulbar haemorrhage.
55 f	Macro:	Right eye with traumatic haemorrhage.
	Micro:	Spleen: minimal focal haemosiderosis.
		Kidneys: slight nephrocalcinosis.
		Right eye: minimal peribulbar haemorrhage.
56 f	Macro:	Right eye with traumatic haemorrhage.
		Lungs with minimal nodules.
	Micro:	Lungs: slight focal collapse.

Compound: TK 10 410

Spleen: minimal focal haemosiderosis.
Liver: moderate irregular fatty changes of the hepatocytes.
Right eye: minimal peribulbar haemorrhage.

57 f Macro: Right eye with traumatic haemorrhage.
Lungs with minimal nodules.
Micro: Lungs: small lymphohistiocytic infiltrates in the interstitium.
Spleen: slight focal haemosiderosis.
Liver: slight irregular fatty changes of the hepatocytes.
Kidneys: minimal nephrocalcinosis.
Right eye: minimal peribulbar haemorrhage.

58 f Macro: Right eye with traumatic haemorrhage.
Lungs slightly mottled.
Micro: Lungs: minute focal congestion.
Spleen: slight focal haemosiderosis.
Liver: moderate irregular fatty changes of the hepatocytes.
Kidneys: minimal nephrocalcinosis.
Right eye: slight peribulbar haemorrhage.

59 f Macro: Right eye with traumatic haemorrhage.
Lungs inflated.
Micro: Lungs: slight focal congestion and haemorrhage.
Spleen: minute focal haemosiderosis.
Liver: minute irregular fatty changes of the hepatocytes. Minute lymphohistiocytic infiltrates mainly in the periportal spaces.
Kidneys: slight nephrocalcinosis.
Right eye: minute peribulbar haemorrhage.

60 f Macro: Right eye with traumatic haemorrhage.
Lungs inflated.
Micro: Lungs: small focal collapse.
Spleen: minute focal haemosiderosis.
Kidneys: minimal nephrocalcinosis.
Right eye: slight peribulbar haemorrhage.

Group 3 - 152 mg/m3

26 m Macro: Right eye with traumatic haemorrhage.
Lungs inflated.
Micro: Lungs: minimal focal collapse.
Liver: minute irregular fatty changes of the hepatocytes.
Muzzle: slight congestion and purulent inflammation with ulceration of the nasal mucosa.

Compound: TK 10 410

Right eye: minimal peribulbar haemorrhage.

27 m Macro: Right eye with haemorrhage.
Lungs inflated.

Micro: Lungs: small areas of focal collapse.
Right eye: slight peribulbar haemorrhage.
Muzzle: slight congestion and purulent inflammation with ulceration of the nasal mucosa.

28 m Macro: Right eye with haemorrhage.
Lungs inflated.

Micro: Lungs: small area of collapse.
Liver: slight irregular fatty changes of the hepatocytes.
Urinary bladder: dilated, with eosinophilic precipitates within the lumen.
Right eye: minimal peribulbar haemorrhage.

29 m Macro: Both eyes with haemorrhage.
Lungs inflated.

Micro: Lungs: small focal recent haemorrhage.
Eyes: minimal peribulbar haemorrhage.
Muzzle: slight congestion, minute purulent inflammation with ulceration of the nasal mucosa.

30 m Macro: Right eye with haemorrhage.
Lungs inflated.

Micro: Lungs: small focal collapse.
Spleen: minimal focal haemosiderosis.
Liver: slight irregular fatty changes of the hepatocytes.
Right eye: no changes seen.
Muzzle: minimal purulent inflammation of the nasal mucosa.

66 f Macro: Right eye with haemorrhage.
Lungs inflated.

Micro: Lungs: small focal collapse.
Spleen: slight focal haemosiderosis.
Liver: slight irregular fatty changes of the hepatocytes.
Right eye: minimal peribulbar haemorrhage.
Muzzle: minimal purulent inflammation of the nasal mucosa.

67 f Macro: Right eye with haemorrhage.
Lungs inflated.
Spleen mottled.

Micro: Lungs: small lymphohistiocytic infiltrates in the interstitium.
Spleen: slight haemosiderosis.
Liver: slight irregular fatty changes of the hepatocytes. Some lymphohistiocytic infiltrates mainly in the periportal spaces.

Compound: TK 10 410

Kidneys: minimal nephrocalcinosis.
Right eye: minimal peribulbar haemorrhage.
Pituitary: not sent for histopathology.

68 f Macro: Right eye with haemorrhage.
Micro: Lungs: small areas of collapse.
Liver: slight irregular fatty changes of the hepatocytes.
Kidneys: minute nephrocalcinosis.
Right eye: minute peribulbar haemorrhage.

69 f Macro: Right eye with haemorrhage.
Lungs inflated.
Micro: Lungs: some lymphohistiocytic infiltrates in the interstitium.
Spleen: minimal focal haemosiderosis.
Kidneys: minimal nephrocalcinosis.
Right eye: minute peribulbar haemorrhage.

70 f Macro: Right eye with haemorrhage.
Lungs with some nodules, slightly inflated.
Micro: Lungs: small areas of collapse.
Spleen: slight haemosiderosis.
Liver: minute irregular fatty changes of the hepatocytes.
Kidneys: minimal nephrocalcinosis.
Right eye: minute peribulbar haemorrhage.
Muzzle: slight congestion and purulent inflammation with ulceration of the nasal mucosa.

Group 4 - 305 mg/m3

31 m - died on the 6th experimental day.
Macro: Stomach: small and large intestine inflated.
Adrenals: with haemorrhages.
Micro: Heart: acute congestion, haemorrhages in the myocardium.
Lungs: focal congestion.
Thymus: depletion of the thymocytes.
Spleen: congestion, haemorrhages.
Stomach: large intestine and small intestine: initial autlysis.
Liver: congestion, small haemorrhages.
Adrenals: congestion, small haemorrhages.
Kidneys: congestion.
Pituitary: congestion, small haemorrhages.
Muzzle: congestion, purulent inflammation with ulceration of the nasal mucosa.
Brain: acute congestion.

Compound: TK 10 410

32 m - died on the 17th experimental day.
Macro: small and large intestine inflated.
Micro: Heart: acute congestion.
Lungs: acute congestion, focal haemorrhages, areas of collapse. Slight accumulation of haemosiderin pigment within some histiocytes and in the interstitium.
Thymus: depletion of the thymocytes.
Spleen: shrunken, slight depletion of the lymphoid tissue.
Liver: acute congestion.
Intestines: initial autolysis.
Adrenals: congestion, recent haemorrhages.
Kidneys: acute congestion.
Testicles: arrest of spermatogenesis at spermatid stage. Large multinucleated spermatids within the lumen of some seminiferous tubules.
Muzzle: congestion, purulent inflammation with ulceration of the nasal mucosa.
Pituitary: acute congestion.
Brain: acute congestion.

33 m - died on the 8th experimental day.
Macro: Large intestine inflated.
Micro: Heart: acute congestion.
Lungs: slight acute congestion, areas of collapse.
Thymus: depletion of the thymocytes.
Adrenals: congestion.
Liver: congestion.
Kidneys: congestion.
Pituitary: congestion.
Muzzle: congestion, minute haemorrhages, minimal inflammatory infiltration in the mucosa.
Brain: slight congestion.

34 m Macro: Both eyes with traumatic haemorrhages.
Lungs and intestines inflated.
Testicles and pituitary small.
Micro: Lungs: small areas of collapse.
Thymus: minute depletion of the thymocytes.
Small and large intestine: no changes seen.
Liver: minute irregular fatty changes of the hepatocytes.
Adrenals: slight congestion.
Testicles: spermatogenesis focally reduced.
Both eyes: minimal peribulbar haemorrhages.
Pituitary: slight congestion.
Muzzle: congestion, purulent inflammation with ulceration of the nasal mucosa.

Compound: TK 10 410

35 m - died on the 12th experimental day.
Macro: Stomach and intestines inflated.
Micro: Heart: congestion, small haemorrhages in the myocardium.
Aorta: not sent for histopathology.
Lungs: congestion, haemorrhagic oedema.
Thymus: depletion of the thymocytes.
Spleen: not sent for histopathology.
Liver: congestion.
Kidneys: slight congestion.
Testicles: spermatogenesis focally reduced.
Muzzle: congestion, purulent inflammation with ulceration of the nasal mucosa.

36 m Macro: Right eye with traumatic haemorrhage.
Lungs with small inflated areas.
Thymus, spleen and testicles small.
Micro: Lungs: recent haemorrhages, slight focal collapse.
Thymus: involution of the thymic tissue.
Spleen: no changes seen.
Testicles: spermatogenesis focally reduced.
Right eye: small peribulbar haemorrhage.
Muzzle: congestion, purulent inflammation with ulceration of the nasal mucosa.

37 m - died after blood collection after the termination of the experiment.
Macro: Right eye with traumatic haemorrhage.
Lungs inflated, stomach and intestines mottled and inflated. Thymus, seminal vesicles, prostate and spleen small.
Micro: Lungs: acute congestion, minute focal recent haemorrhage.
Thymus: not sent for histopathology.
Spleen: shrunken, depletion of the lymphoid tissue.
Stomach, intestines: initial autolysis.
Liver: slight congestion.
Kidneys: slight congestion.
Seminal vesicles, prostate: no changes seen.
Muscle: not sent for histopathology.
Right eye: no changes seen.
Muzzle: congestion, purulent inflammation with ulceration of the nasal mucosa.
Brain: lateral ventricles dilated.

38 m - died on the 9th experimental day.
Macro: Stomach and intestines: inflated.
Micro: Heart: congestion, small haemorrhages in the myocardium.
Lungs: congestion, focal haemorrhages, areas of desquamative pneumonia.
Thymus: congestion, haemorrhages, depletion of the thymocytes.

Compound: TK 10 410

Spleen: congestion.
Stomach and intestines: autolysis.
Liver: congestion.
Adrenals: congestion, haemorrhages.
Kidneys: congestion focal haemorrhages, autolysis.
Testicles: arrest of spermatogenesis at spermatid stage. Large multinucleated spermatids within the lumen of some seminiferous tubules.
Pituitary: congestion.
Muzzle: purulent inflammation with ulceration in one place of the nasal mucosa.

39 m Macro: Right eye with traumatic haemorrhages.
Lungs mottled, spleen and thymus small.
Intestines inflated.
Micro: Heart: slight congestion.
Lungs: minute areas of collapse.
Spleen: no changes seen.
Thymus: minimal depletion of the thymocytes.
Intestines: no changes seen.
Liver: slight irregular fatty changes of the hepatocytes.
Right eye: small peribulbar haemorrhage.
Muzzle: congestion, purulent inflammation with ulceration of the nasal mucosa.

40 m - died on the 17th experimental day.
Macro: Intestines and stomach inflated.
Micro: Heart: haemorrhages in the myocardium, congestion.
Lungs: congestion, minute focal haemorrhages.
Thymus: slight depletion of the thymocytes.
Stomach and intestines: autolysis.
Liver: acute congestion.
Pancreas: focal autolysis.
Adrenals: slight congestion.
Kidneys: acute congestion.
Pituitary: congestion, minute haemorrhages.
Muzzle: congestion, purulent inflammation with ulceration of the nasal mucosa.

71 f - died on the 21st experimental day.
Macro: Lungs mottled, left ovary with haemorrhages.
Stomach and intestines inflated.
Micro: Heart: congestion, minute haemorrhages in the myocardium.
Lungs: congestion, haemorrhagic oedema.
Lymph nodes (abdominal): congestion, marked haemorrhages.
Stomach and intestines: autolysis.
Thymus: depletion of the thymocytes.
Liver: congestion, marked haemorrhages.

Compound: TK 10 410

Adrenals: congestion, marked haemorrhages.
Kidneys: congestion, minute haemorrhages. Slight nephrocalcinosis, autolysis.
Left ovary: congestion, haemorrhages in corpora lutea and interstitium.
Right ovary: not sent for histopathology.
Pituitary: congestion.
Muzzle: congestion, purulent inflammation with ulceration of the nasal mucosa.
Brain: slight acute congestion.

72 f - died on the 8th experimental day.
Macro: Stomach and intestines inflated.
Micro: Heart: congestion, haemorrhages in the myocardium.
Lungs: congestion, marked haemorrhagic oedema.
Thymus: some vessels dilated, marked depletion of the thymocytes.
Stomach: autolysis.
Intestines: autolysis.
Adrenals: congestion.
Liver: congestion, minute haemorrhages.
Kidneys: congestion, minimal nephrocalcinosis.
Ovaries: congestion, moderate haemorrhages.
Pituitary: congestion.
Muzzle: congestion, purulent inflammation with ulceration of the nasal mucosa.

73 f - died on the 10th experimental day.
Macro: Stomach and small intestine inflated.
Micro: Lungs: congestion, marked haemorrhagic oedema.
Thymus: congestion, depletion of the thymocytes.
Spleen: shrunken, atrophy of the lymphoid tissue.
Stomach: autolysis.
Intestines: autolysis.
Liver: congestion, marked haemorrhages.
Adrenals: congestion, haemorrhages.
Kidneys: congestion, slight haemorrhages.
Ovaries: congestion, minute haemorrhages.
Pituitary: congestion.
Muzzle: congestion, purulent inflammation with ulceration of the nasal mucosa.

74 f - died on the 12th experimental day.
Macro: Stomach and small intestine inflated.
Right eye: small haemorrhage.
Micro: Heart: congestion, moderate haemorrhages in the myocardium.
Lungs: moderate congestion and haemorrhagic oedema.
Thymus: congestion, depletion of the thymocytes.
Spleen: shrunken, atrophy of the lymphoid tissue.
Stomach and small intestine: autolysis.
Liver: minute congestion.

Compound: TK 10 410

Kidneys: slight congestion, autolysis.
Ovaries: dilatation of the blood vessels, minute haemorrhages.
Right eye: slight peribulbar haemorrhage.
Pituitary: congestion.
Muzzle: congestion, purulent inflammation with ulceration of the nasal mucosa.

75 f Macro: Right eye with traumatic haemorrhage.
Lungs and intestines inflated, thymus and left ovary small.
Micro: Lungs: slight focal collapse.
Thymus: slight depletion of the thymocytes.
Spleen: minimal focal haemosiderosis.
Intestines: no changes seen.
Liver: minimal congestion.
Kidneys: minimal congestion, minimal nephrocalcinosis.
Left ovary: no changes seen.
Muzzle: congestion, purulent inflammation with ulceration of the nasal mucosa.
Right eye: no changes seen.

76 f - died on the 8th experimental day.
Macro: Stomach and intestines inflated.
Micro: Lungs: congestion, minute haemorrhages.
Thymus: congestion, depletion of the thymocytes.
Stomach and intestines: no changes seen.
Liver: marked congestion, minute haemorrhages.
Kidneys: slight congestion.
Ovaries: congestion.
Muzzle: congestion, purulent inflammation with ulceration of the nasal mucosa and of the skin of the muzzle.
Pituitary: congestion.

77 f - died on the 6th experimental day.
Macro: Stomach and small intestine inflated.
Micro: Heart: congestion. Minute haemorrhages in the myocardium.
Thymus: depletion of the thymocytes.
Lungs: congestion, haemorrhagic oedema.
Spleen: shrunken, slight atrophy of the lymphoid tissue.
Stomach, small intestine: autolysis.
Liver: marked congestion, haemorrhages.
Kidneys: minute nephrocalcinosis, congestion.
Pituitary: congestion.
Muzzle: congestion, purulent inflammation with ulceration of the nasal mucosa and of the skin.

Compound: TK 10 410

78 f - died on the 8th experimental day.
Macro: Stomach and small intestine inflated.
Micro: Heart: congestion, minute haemorrhages in the myocardium.
Lungs: marked congestion and haemorrhagic oedema.
Thymus: depletion of the thymocytes.
Spleen: minute focal haemosiderosis, shrunken. Slight atrophy of the lymphoid tissue.
Stomach, small intestine: autolysed.
Liver: moderate congestion, minute haemorrhages.
Adrenals: slight congestion.
Kidneys: minimal nephrocalcinosis, congestion.
Ovaries: congestion.
Pituitary: congestion.
Muzzle: congestion, purulent inflammation with ulceration of the nasal mucosa.
Brain: acute congestion.

79 f - died on the 7th experimental day.
Macro: Stomach and small intestine inflated. Large intestine with haemorrhages. Left eye with haemorrhages, right eye damaged during section.
Micro: Heart: congestion, minimal haemorrhages.
Lungs: marked congestion and haemorrhagic oedema.
Thymus: depletion of the thymocytes.
Stomach and intestines: autolysed.
Liver: slight congestion.
Kidneys: slight nephrocalcinosis, congestion, autolysis.
One ovary: congestion, haemorrhages.
Pituitary: congestion.
Eyes: traumatic lesion.
Muzzle: congestion, purulent inflammation with ulceration of the nasal mucosa.

80 f - died on the 7th experimental day.
Macro: Stomach and intestines inflated.
Both eyes with heamorrhages.
Micro: Heart: congestion, small haemorrhage in the myocardium.
Lungs: minute recent focal haemorrhage.
Thymus: marked depletion of the thymocytes.
Stomach and small intestine: no change seen.
Large intestine: not sent for histopathology.
Liver: slight irregular fatty changes of the hepatocytes, congestion, slight haemorrhages.
Kidneys: congestion, minute haemorrhages.
Ovaries: congestion, small haemorrhages.
Pituitary: congestion.
Muzzle: congestion, purulent inflammation with ulceration of the nasal mucosa and of the skin.
Eyes: minute peribulbar haemorrhage.

Compound: TK 10 410
Hist.No.: Ex 495

Group 1 - Controls + Recovery Period of 21 Days

1 m Macro: no changes seen.
Micro: Lungs: small areas of lymphohistiocytic infiltrates in the interstitium.
Spleen: minute extramedullary haematopoiesis.
Liver: minute lymphohistiocytic infiltrates mainly in the periportal spaces. Slight irregular fatty changes of the hepatocytes.
Urinary bladder: dilated, with eosinophilic precipitates within the lumen.

2 m Macro: no changes seen.
Micro: Spleen: minute extramedullary haematopoiesis. Minimal focal haemosiderosis.
Liver: minute irregular fatty changes of the hepatocytes.
Urinary bladder: dilated, with eosinophilic precipitates within the lumen.

3 m Macro: no changes seen.
Micro: Lungs: minute recent haemorrhages. Some lymphohistiocytic infiltrates in the interstitium.
Spleen: minute extramedullary haematopoiesis and minute focal haemosiderosis.

4 m Macro: no changes seen.
Micro: Lungs: small recent haemorrhages.
Spleen: slight extramedullary haematopoiesis and minimal focal haemosiderosis.
Liver: slight irregular fatty changes of the hepatocytes.
Urinary bladder: dilated, with eosinophilic precipitates within the lumen.

5 m Macro: no changes seen.
Micro: Lungs: small areas of collapse.
Spleen: minute focal haemosiderosis.

41 f Macro: no changes seen.
Micro: Lungs: areas of collapse.
Spleen: minimal extramedullary haematopoiesis.
Kidneys: minimal nephrocalcinosis.

42 f Macro: no changes seen.
Micro: Lungs: small recent haemorrhages. Some lymphohistiocytic infiltrates in the interstitium.
Spleen: moderate focal haemosiderosis.
Kidneys: minute nephrocalcionosis.

43 f Macro: no changes seen.
Micro: Lungs: small recent haemorrhages.
Spleen: minute extramedullary haematopoiesis, slight focal haemosiderosis.
Kidneys: minimal nephrocalcinosis.

Compound: TK 10 410

44 f Macro: no changes seen.
Micro: Lungs: small recent haemorrhage, small areas of collapse.
Spleen: minute focal haemosiderosis.
45 f Macro: no changes seen.
Micro: Lungs: small areas of collapse.
Spleen: minute extramedullary haematopoiesis and slight focal haemosiderosis.
Kidneys: minute nephrocalcinosis.

Group 3 - 152 mg/m3 + Recovery Period of 21 Days.

21 m Macro: Lungs inflated, seminal vesicle small, brain mottled and enlarged.
Micro: Lungs: minimal recent haemorrhage and some lymphohistiocytic infiltrates in the interstitium.
Spleen: minute focal haemosiderosis.
Urinary bladder: dilated, with eosinophilic precipitates within the lumen.
Seminal vesicles: no changes seen.
Brain: marked enlargement of both lateral ventricles.
22 m Macro: no changes seen.
Micro: Lungs: small recent haemorrhage.
Spleen: slight extramedullary haematopoiesis.
Urinary bladder: dilated, with eosinophilic precipitates within the lumen.
23 m Macro: no changes seen.
Micro: Lungs: some focal recent haemorrhages. Some lymphohistiocytic infiltrates in the interstitium.
Spleen: minimal focal haemosiderosis, minute extramedullary haematopoiesis.
Liver: minute irregular fatty changes of the hepatocytes, few lymphohistiocytic infiltrates in the interstitium.
Muzzle: in one place remnants of partially re-epithelized ulcus showing foreign body granuloma and chronic inflammatory infiltration in the base.
24 m Macro: no changes seen.
Micro: Lungs: small recent haemorrhages, minute lymphohistiocytic infiltrates in the interstitium.
Spleen: minute extramedullary haematopoiesis.
Liver: minimal irregular fatty changes of the hepatocytes.

Compound: TK 10 410

25 m Macro: Right testicle small.
Micro: Lungs: small areas of lymphohistiocytic infiltrates in the interstitium.
Spleen: minute extramedullary haematopoiesis.
Liver: minute irregular fatty changes of the hepatocytes.
Right testicle: complete arrest of spermatogenesis, marked atrophy of the seminiferous tubules.

61 f Macro: no changes seen.
Micro: Lungs: small areas of collapse.
Spleen: minute extramedullary haematopoiesis, slight focal haemosiderosis.
Kidneys: minute nephracalcinosis.

62 f Macro: no changes seen.
Micro: Lungs: some lymphohistiocytic infiltrates in the interstitium.
Spleen: slight focal haemosiderosis.
Kidneys: minute nephrocalcinosis.

63 f Macro: no changes seen.
Micro: Spleen: minute extramedullary haematopoiesis, slight focal haemosiderosis.
Kidneys: minute nephrocalcinosis.

64 f Macro: Lungs inflated.
Micro: Lungs: small recent haemorrhages.
Spleen: minute extramedullary haematopoiesis, minimal focal haemosiderosis.
Kidneys: minute nephrocalcionosis.

65 f Macro: no changes seen.
Micro: Lungs: small areas of collapse.
Spleen: minimal extramedullary haematopoiesis, slight focal haemosiderosis.
Kidneys: minute nephrocalcinosis.

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Industrial Medicine
THE UNIVERSITY OF TEXAS MEDICAL BRANCH
GALVESTON, TEXAS 77550

DEC 5 1977

I.D. # 10766 AMES

Department of Preventive Medicine and Community Health
Division of Environmental Toxicology and Epidemiology

November 15, 1977

I.D. # 10767 Mouse/Oral Body Fluids

I.D. # 10769 Mouse Dermal Dominant-Lethal

I.D. # 11032 Human/DNA Repair

REPORT TO THE DOW CHEMICAL COMPANY

INTEGRATED MUTAGENICITY TESTING PROGRAM

I.D. # 10765 Salmonella/Host Med. Assy

I.D. # 11031 Mouse Micronucleus

PERMANENT RECORD FILE
INDUSTRIAL MEDICINE

Prepared by: Terry Pullin

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Marvin S. Legator, Ph.D.
Professor and Director

INTRODUCTION

Exposure to toxic chemicals in our environment may be one of the major contributing factors to the mutagenic burden in man. Some man-made chemicals currently in widespread use in the population have been found to be mutagenic in sub-human experimental systems suggesting that such chemicals constitute a potential genetic hazard for man. The risk poses itself to both present and future generations; to the former, as the production of genetic lesions in somatic cells, possibly leading to cancer; while to the latter as the production of transmissible gene mutations and alterations in chromosome structure and number.

Several classes of these man-made compounds have been shown to have the ability of inducing mutations. The work of Rapoport[1] revealed that many alkylating agents are mutagenic, some of them with great potency. Haddow[2] pointed out that certain diepoxy compounds active as cross-linking agents may lead to biological alkylation. Hendy and his co-workers[3] found both radiomimetic and cytotoxic activity in their series of bisepoxides. Hine and Rowe[4] have stated that the majority of observations seem to indicate that the physiological activity of the epoxides is in three areas:

(1) central nervous system depression

(2) irritation of surface tissues and

(3) radiomimetic action.

In recognition of a potential hazard that exists concerning epoxide induced genetic damage, The Dow Chemical Company submitted two epoxy resins and five epoxy diluents to Dr. Harvin S. Legator's Laboratory, at the University of Texas Medical Branch for assessment of their mutagenic activity by means of a battery of mutagenic

SUMMARY

Several epoxides were evaluated for mutagenic activity by the following test systems: (a) microbial mutagenic assay system, (b) body fluid analysis, (c) host mediated assay, (d) induction of DNA repair, (e) micronucleus test and (f) the dominant lethal test.

DGEDPA (diglycidyl ether bisphenol A), DGEDPA (distilled) and DCPDGE (dicyclopentadiene glycidyl ether) were mutagenic in the microbial assay but inactive in the remainder of the test systems. In view of the inactivity in the intact animal systems, it is felt that these compounds pose the least mutagenic hazard to man of the chemicals evaluated.

DGENPG (diglycidyl ether of neopentyl glycol) and CGE (o-cresyl glycidyl ether) were mutagenic in bacteria and induced DNA repair. In addition, mutagenicity was discovered in the urine of DGENPG and CGE treated mice. These results plus a possibility of preimplantation loss in the dominant lethal test suggest that DGENPG and CGE are weak mutagens.

BGE (butyl glycidyl ether) was determined to be mutagenic as evidenced by an induction of increased dominant lethality, DNA repair and bacterial mutation. These data also support the conclusion that BGE poses a hazard by skin exposure.

The C_{12}-C_{14} alkyl glycidyl ether was only active in the microbial assay with very minimal activity. This data leads to the determination of the C_{12}-C_{14} alkyl glycidyl ether as nonmutagenic.

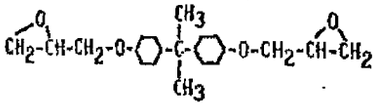
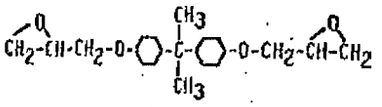
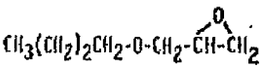
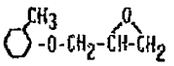
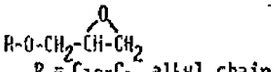
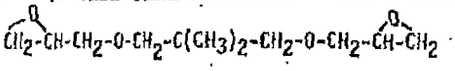
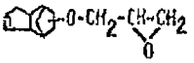
tests. These test chemicals are some of the major epoxides currently used in industry and have a potential widespread exposure to the population.

The investigation of these epoxides consisted of a comprehensive protocol involving several test systems thereby extending coverage far beyond that achieved in virtually any other test program.

MATERIALS AND METHODS

The epoxides submitted for mutagenic evaluation are shown in Table 1, and were assayed in the following test systems: (a) the microbial mutagenic test system, the well known "Ames System", (b) body fluid analysis, (c) host mediated assay, (d) induction of DNA repair, (e) the micronucleus test and (f) the dominant lethal test. The methodologies of these various test systems are shown in the following paragraphs.

TABLE 1
EPOXIDES STUDIED BY BATTERY OF MUTAGENIC TESTS

CHEMICAL	STRUCTURE
RESINS	
1. Diglycidyl ether of 2,2-di(p,p'-hydroxyphenyl)propane (DGEBPA)	
2. Diglycidyl ether of 2,2-di(p,p'-hydroxyphenyl)propane (DGEBPA) Distilled	
DILUENTS	
1. Butyl Glycidyl Ether (BGE)	
2. o-Cresyl Glycidyl Ether (CGE)	
3. Alkyl Glycidyl Ether (Alkyl GE)	 R = C_{12}-C_{14} alkyl chain
4. Diglycidyl Ether of Neopentyl Glycol (DGENPG)	
5. Dicyclopentadiene Glycidyl Ether (DCPDGE)	

Microbial Test System. The epoxy resins and diluents were tested directly against strains TA1535 and TA98 of Salmonella typhimurium according to the methods of Ames et al.[5] "S-9" liver homogenate fraction was prepared from liver of phenobarbital-induced and Aroclor-induced rats, and a liver homogenate mix in the amount of 0.5 ml was used in the appropriate test plates as shown in Table 2. The liver homogenate mix contained S-9 fraction, 0.3 ml; $MgCl_2$8mM; KCl, 33mM; glucose-6-phosphate, 5mM; NADP, 4mM; and sodium phosphate (pH 7.4), 100mM.

Petri plates containing 20 ml minimal glucose agar medium (consisting of Spizizen minimal medium supplemented with 0.5 µg/ml biotin and 4 µg/ml histidine) were used for the test. Two ml of molten top agar were mixed with 0.1 ml of nutrient broth cultures of the tester strain that were grown overnight. This was then mixed with the epoxy resin or diluents. The concentration of each of these resins or diluents is shown in Table 2.

The petri plates were incubated at 37°C for 48 hours. Results were expressed as the ratio of the number of bacterial colonies (revertants to histidine prototrophy) on the plates treated with the various chemicals to the number of colonies (spontaneous revertants) on the control plates. Results are considered to be negative if the difference in the number of induced revertants compared to the number of spontaneous revertants is less than two-fold.

Body Fluid Analysis. Groups of ten $B_6D_2F_1$ female mice, 20-25 g and female ICR, 25-35 g were each given an oral dose of the resin or diluent in corn oil once a day for four days. The dosages are shown in Tables 3 and 4 for each resin or diluent. Following the treatment each group of mice was placed in a separate metabolism cage, and urine was collected in a chilled container for 24 hours. The urine was then

directly tested against TA1535 with and without the addition of β-glucuronidase (Tables 3 and 4) according to the procedure of Durston and Ames[6].

Micronucleus Test. The micronucleus test, as described by Schmid[7] was conducted on each of the diluents or resins. In addition, triethylmelanine (TEM) was used in this test as a positive control. Each compound was administered orally once a day for five days to separate groups of ten $B_6D_2F_1$ female mice, 20-25 g, in varying dosages as seen in Table 6. TEM was injected intraperitoneally at a dosage of 0.05 mg/kg. All animals were sacrificed 4 hours after the last treatment. Extraction of bone marrow, preparation of smears, staining, and analysis of slides was then performed as described by Schmid[7]. Statistical analysis of the data from this test consisted of comparisons between the epoxide groups of mice and the control group by use of Dunnett's "t" test.

Induction of DNA Repair. The epoxides were examined for induction of excision repair measured as unscheduled DNA-synthesis in human mononucleated white cells (G-0 phase) in a short-term in vitro assay using liquid scintillation spectroscopy. Approximately 1.4 million white cells were added to freshly prepared culture medium (Eagle's minimum essential medium + 15% fetal calf serum + 2mM glutamine - Gibco) followed by either 1, 10, 100 or 500 ppm of the test compound. However, CGE was tested at 10, 100 and 1000 ppm. These concentrations ranged between 100% cell viability and the toxic dose determined on cultured cells following an in vitro exposure of 4.5 hours. The test compounds were dissolved in and diluted with dimethylsulfoxide (DMSO) prior to addition to the white cells and were added to triplicate samples of 0.1 ml quantities. Positive control samples received

4-nitroquinoline-1-oxide (4-NQO) in saline at a final concentration of 4 μM in 0.1 quantities. Immediately following the addition of each epoxide to the culture medium containing the white cells, the samples were incubated for 5 hours at 37°C.

About 30 minutes prior to harvesting the cells, the cell viability was determined by trypan blue exclusion. Following this, the cells were collected on glass fiber filters, extensively rinsed with distilled water and air-dried. Filters were subsequently placed in vials containing seven ml of toluene-PPO-POPOP mixture for liquid scintillation counts. Amount of tritiated thymidine (^{3}H-TdR) incorporation was expressed in counts per minute.

For radioautographic studies, the white cells were centrifuged and the supernatant removed following incubation. Cells were subsequently suspended in hypotonic KCl solution, exposed to methanol: glacial acetic acid (4:1) fixative, dropped on slides and allowed to air dry. The slides were then dipped in a Kodak NTB$_2$: distilled water emulsion, exposed at 4°C for 10 days and developed in Kodak D-19. They were then stained with Giemsa stain, and grain counts were performed over 400 nuclei per concentration. Statistical analysis of this data consisted of Duncan's Least Significant Difference Test (LSD) performed at the 0.05 level. A value less than .05 indicates a significant difference between the control cells and the chemically treated cells.

Host Mediated Assay. The Salmonella strains were maintained on Standard Methods agar slants and transferred into Nutrient broth. For enumeration of his+ revertants, Spizizen's minimal medium was used, supplemented with 0.5 μg/ml biotin and 4.0 μg histidine/ml to allow several divisions, necessary in detecting certain mutagenic agents.

The bacteria were grown to a density of approximately 2×10^7 cells/ml two hours prior to sacrifice of the animals (5th day of treatment; log phase). A 23 gauge needle was used to inoculate 2.0 ml of this cell suspension into the peritoneal cavity of female ICR: albino mice (after the area of inoculation had been swabbed with ethanol). Mice weighed 25-30 g and were maintained on a diet of Purina Mouse Feed or equivalent. The experiment was allowed to run for six hours after inoculation of the bacteria suspension.

The abdominal regions of the mice were swabbed with ethanol, and 2 ml of sterile saline was injected into the peritoneal cavity of each mouse. The exudate was withdrawn using a tuberculine syringe with a 19 gauge needle. The peritoneal exudates from each mouse were then run separated.

Dilution blanks, containing 4.5 ml of sterile saline were prepared in advance. Tenfold serial dilutions were made of each peritoneal exudate (0.5 ml + 4.5 ml) yielding a concentration series from 10^0 (undiluted peritoneal exudate) through 10^{-6}.

For enumeration of the total bacterial counts, the 10^{-4} - 10^{-6} dilutions were plated on Standard-Methods agar: three plates per sample, 0.2 ml of sample per plate. Each sample was spread over the surface of the plate with a bent glass rod immersed in 95% ethanol and flamed just prior to use.

In plating for the total mutant counts on minimal agar, the 10^0 dilution was used. With the 10^0 dilution, two plates were used (0.20 ml sample/plate). All plates were incubated at 37°C. The Standard Methods agar plates were incubated for 24 hours and the minimal media for 48 hours.

Scoring in this assay was accomplished in the following manner: No. colonies per platex 6 = colony-forming units (CFU) per ml of sample plated. CFU/ml x 1/dilution factor (10^{-4} - 10^{-5}) = CFU/ml in diluted exudates. The mutation frequency (MF) is calculated for each sample.

$$MF = \frac{\text{His+ revertants/ml}}{\text{CFU/ml in undiluted exudate}}$$

$$MFt/MFc = \frac{\text{MF of experimental sample}}{\text{MF of control sample}}$$

MFt/MFc = 1.00 for control sample

t = treated; c = control

Dominant Lethal Assay. All experiments were carried out with $D_6D_2F_1$ hybrid mice; males were 8-10 weeks old at the commencement of experiments and females 8-10 weeks old when mated.

The epoxy resins and diluents were applied topically in the undiluted form. Triethylenemelamine (TEM) was obtained from the FDA and prepared freshly in 0.9% saline.

Since there was no data available regarding the percutaneous toxicity of these epoxies in mice it was necessary to conduct range-finding toxicity studies on each epoxide for 4-6 weeks to determine the dosage and surface area on the mice to be used in this study. The dosages for each epoxide determined from this range finding study were:

DGEBPA	~3.0 g/k
DGEBPA (Distilled)	~3.0 g/k
BGE	1.5 g/k
CGE	1.5 g/k

Alkyl GE	2.0 g/kg
DGENPG	1.5 g/kg
DCPDGE	2.0 g/kg

Previous to the start of the dominant lethal assay, three untreated virgin females were randomly caged with one untreated male for a week. At the end of the week the three females were replaced by three other untreated virgin females which remained with the untreated male for the second week. The data collected from each untreated male during the two weeks of breeding prior to use in the dominant lethal established the fertility of the male and provided background data regarding litter size, spontaneous fetal deaths, etc. This data could also serve as a control for each individual male.

Following the collection of the two weeks breeding data, the dominant lethal assay was initiated. The modified procedure of Green et al[8] was used. A minimum of ten males of proven fertility were randomly selected for each epoxide. In addition, a negative control consisting of topically applied 0.9% saline and a positive control of TEM treated males was also used in this study. Males selected for the topical applications were prepared in the following manner: approximately 15-20% of the surface area of these mice were clipped by electric shears in the dorsal area; following this, the remaining hair on the clipped area was chemically depilated (Neet, Whitehall Labs, Inc.) so that no hair remained to interfere with absorption of the chemical. Chemical depilation was only used as needed following the initial removal of hair and did not exceed one dipilation per week. A minimum of 24 hours was allowed between

chemical depilation and application of the test chemical. Following this preparation, each male was treated with the dosage, as determined by the range-finding toxicity study, of the appropriate chemical three times per week for a minimum of eight weeks. Each group of males for each epoxide was weighed weekly, and the mean weight for each week was used to adjust the dosage so that the appropriate dose was maintained throughout the treatment period. Following the treatment period, three untreated virgin females were randomly caged per treated male for one week. At the end of the first week the females were replaced with three other untreated virgin females for the duration of the second week.

The positive control males were injected I.P. once with 0.2 mg/kg TEM. The week following the injection, three untreated virgin females were randomly caged with each of these males for one week. These females were then replaced with three other virgin females per male for the second week.

All females were sacrificed by cervical dislocation 13-14 days from the midweek of the caging and presumptive mating, without being checked for vaginal plugs. At autopsy, females were scored for pregnancy, total number of implants and fetal deaths.

The variables taken into account in this assay included: total number implants/pregnancy, proportion fetal deaths/pregnancy and % pregnant females. Comparisons were made between the epoxide groups of mice and control group of mice by analysis of variance.

RESULTS

The results are arranged by the individual epoxides in order to facilitate ease of comprehension of the overall mutagenicity of each epoxy. A table of results of each test for each epoxide is presented at the end of this section.

be readily seen in Figure 10. There again appeared, as in most epoxides, a significant effect in the number implants/pregnancy due to time ($p=.04$) and time-treated dependency ($p=.0001$).

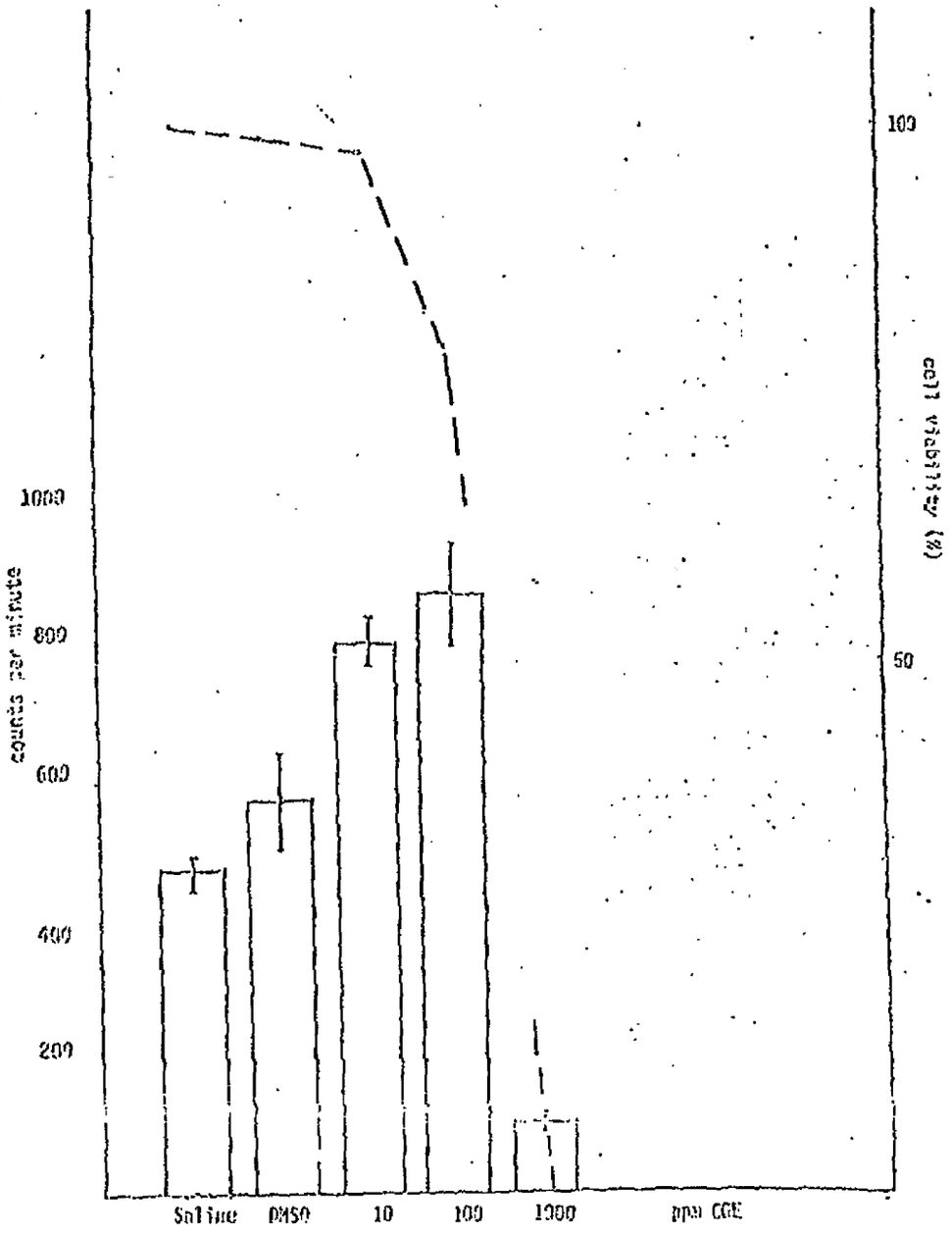


Figure 9. The effect of o-Cresyl Glycidyl Ether (CGE) on tritiated thymidine incorporation into unstimulated, human, mononucleated white cells and the effect on cell viability (broken line) following in vitro

Table 17

Mean Number of Implants/Pregnancy, Proportion Deaths/Pregnancy and % Pregnancy For CGE By Time

		Pretreatment	1 week Post Treatment	2 weeks Post Treatment
CGE	Number of Implants/ Pregnancy	7.614	7.348	6.970
	Proportion Deaths/ Pregnancy	.020	.030	.029
	% Pregnancy	51.515	75.818	63.636
Control	Number of Implants/ Pregnancy	7.077	6.662	8.277
	Proportion Deaths/ Pregnancy	.010	.023	.020
	% Pregnancy	78.100	73.400	83.500

Table 18

Significance Level (p)* of Effects Due To Treatments, Time, and Time-Treatment Dependency

	Analysis of Variable		
	Proportion Deaths/Pregnancy	Number Implants/Pregnancy	% Pregnancy
Effect of:	p	p	p
CGE vs control (Treatments)	.45	.86	.03
Time	.79	.04	.41
CGE by Time	.99	.0001	.18

* Significance level derived using two factor analysis of variance with repeated measures on factor B. Factor A = treatment. Factor B = time. (reference: Statistical Principles In Experimental Design. B.J. Winer, 2nd Edition).

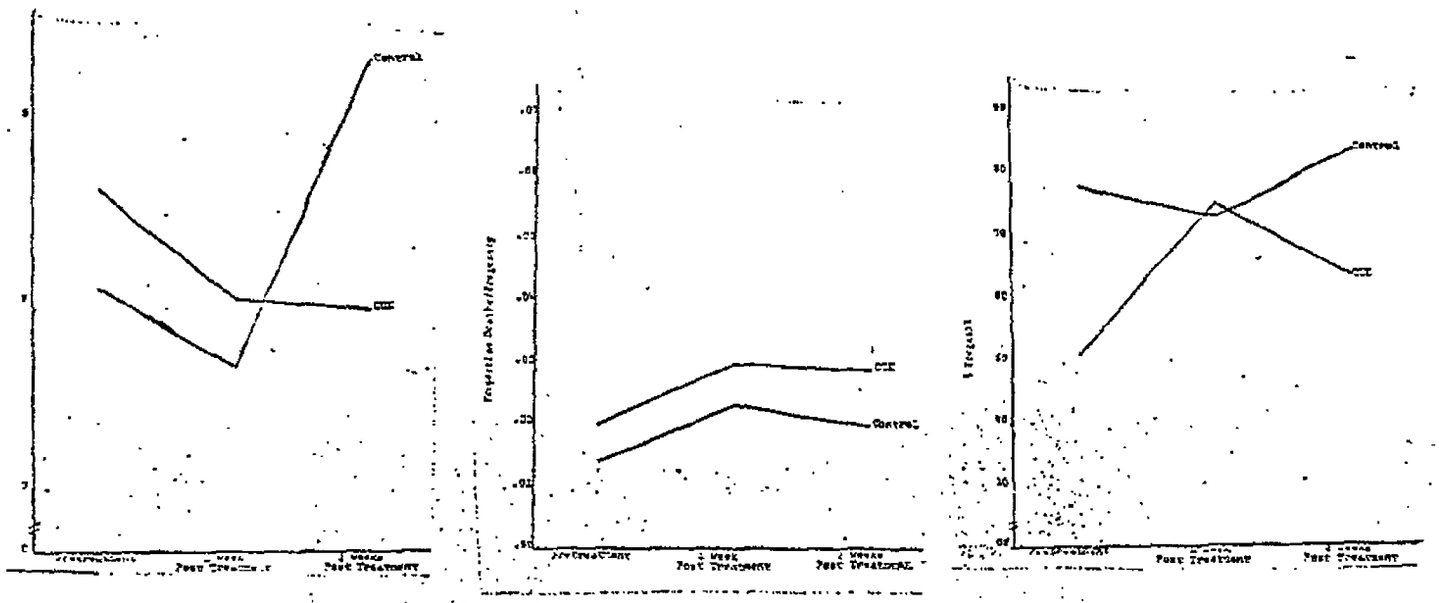


Figure 10. Comparison between CGE and control in the three variables measured in dominant lethal test.

ALKYL GLYCIDYL ETHER (ALKYL GE). This chemical was the least active of the epoxides tested in the direct in vitro microbial assay. It should be noted that it only exhibited mutagenic activity at the highest dose with Aroclor metabolic activation.

The R_t/R_c values determined from the mutagenic analysis of the urine of the DDF mice treated with Alkyl GE ranged from 1.38 without the use of β-glucuronidase to 1.11 with the use of this enzyme (Table 3). The values with the ICR mice ranged from 1.21 to 1.08. These values were deemed insignificant in this test system.

An elevation of the mutation frequency was detected in the host-mediated assay with this epoxide. However, as found in DGEBPA and DGEMPG a decreased growth of the microorganisms in the animals also occurred. Thus it is felt the elevated mutation is not an effect of the Alkyl GE but rather a reflection of the poor bacterial growth.

Alkyl GE gave almost identical micronuclei values as the control (Table 6), and thus this chemical did not produce a mutagenic effect in terms of micronuclei at the dosage tested.

There was no increase in unscheduled DNA synthesis induced by the Alkyl GE over the control as illustrated in Figure 11. Cell viability was extremely low at 100 ppm and nonexistant at 500 ppm. These findings were likely responsible for the significant decreases in ^{3}H-thymidine incorporation at the 100 and 500 ppm doses.

Although the proportion deaths/pregnancy for the Alkyl GE increased steadily with time, these values only ranged from 0.4% to 1.1% (Table 19) and were significantly lower ($p=.003$, Table 20) than the control values. There were no differences between the control and this epoxide in % pregnant as depicted in Figure 12. However, the ever present significant effects on the number implants/pregnancy due to time and time-treatment dependency were present in the Alkyl GE also.

CD 5/97/170

10883

MUTAGENICITY EVALUATION OF

Ka-61

IN THE AMES SALMONELLA/MICROSOME PLATE TEST

FINAL REPORT

SUBMITTED TO:

CIBA-GEIGY
ARDSLEY, NEW YORK 10502

MA-61=(ARALDITE) DY 028
Batch # N 281

SUBMITTED BY:

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20795

IBI PROJECT NO. 20838

MARCH 1978

Litton BIONETICS

RECEIVED
OCT

1/11

I. SPONSOR: Ciba-Geigy

II. MATERIAL*

A. Identification: Ma-61

B. Date Received: January 9, 1978

C. Physical Description: Clear yellow liquid

III. TYPE OF ASSAY: Ames Salmonella/Microsome Plate Assay

IV. PROTOCOL NO.: CMT-100

V. RESULTS

The results of this assay are presented in Table 1.

VI. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound was examined for mutagenic activity in a series of in vitro microbial assays employing Salmonella and Saccharomyces indicator organisms. The compound was tested directly and in the presence of liver microsomal enzyme preparations from Aroclor-induced rats.

The compound was tested over a series of concentrations such that there was either quantitative or qualitative evidence of some chemically-induced physiological effects at the high dose level. The low dose in all cases was below a concentration that demonstrated any toxic effect. The dose range employed for the evaluation of this compound was from 0.001 µl to 5 µl per plate. The compound was toxic to all the strains at 5 µl per plate.

The results of the tests conducted on the compound in the absence of a metabolic system were positive with the strains TA-1535 and TA-100. The test with TA-1535, TA-98 and TA-100 were repeated at all doses because these strains exhibited increased revertants in the initial test. The repeat tests were positive with TA-1535 and TA-100 and negative with TA-98.

The results of the tests conducted on the compound in the presence of the rat liver activation system were positive with the strain TA-1535. The test with TA-100 was repeated because this strain exhibited increased revertants at 1 µl dose level in the initial test. The repeat test was negative.

*Information was supplied by the sponsor. If information was not indicated by the sponsor, N.I. was entered.

VI. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound, Na-61, exhibited genetic activity with TA-1535 strain in activation and nonactivation assays and with TA-100 in the nonactivation assays, conducted in this evaluation and is considered as mutagenic under these test conditions.

Submitted by:

Study Director

D.R. Jagannath 4.6.78

D.R. Jagannath, Ph.D. Date
Section Chief
Submammalian Genetics
Department of Molecular
Toxicology

Reviewed by:

4/6/78

David J. Brusick, Ph.D. Date
Director
Department of Molecular
Toxicology

V. RESULTS

TABLE 1

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: NA-A1
B. SOLVENT: DMSO
C. TEST INITIATION DATE: JAN. 12, 1978
NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS (UL) OR MICROGRAMS (UG) PER PLATE.

TEST	SPECIES	TISSUE	REVERTANTS PER PLATE									
			TA-1535		TA-1537		TA-1538		TA-98		TA-100		DA*	
			1	2	1	2	1	2	1	2	1	2	1	2
NONACTIVATION														
SOLVENT CONTROL	---	---	30	11	20		20		42	38	136	128	43	
POSITIVE CONTROL**	---	---	2115	810	474		2065		2218	1777	2029	1263	832	
TEST COMPOUND														
0.001000 UL	---	---	21	22	12		26		72	29	103	129	61	
0.010000 UL	---	---	131	95	16		17		50	37	134	161	57	
0.100000 UL	---	---	375	140	30		24		50	41	251	281	50	
1.000000 UL	---	---	595	218	9		14		92	15	411	553	58	
5.000000 UL	---	---	104	0	0		0		0	0	0	0	6	
ACTIVATION														
SOLVENT CONTROL	RAT	LIVER	27		8		17		32		241	203	159	
POSITIVE CONTROL***	RAT	LIVER	302		271		1029		1456		1619	--	1904	
TEST COMPOUND														
0.001000 UL	RAT	LIVER	20		8		15		31		211	--	173	
0.010000 UL	RAT	LIVER	29		9		13		25		189	--	161	
0.100000 UL	RAT	LIVER	90		8		17		24		243	271	183	
1.000000 UL	RAT	LIVER	237		9		17		37		381	486	151	
5.000000 UL	RAT	LIVER	71		7		6		37		282	296	109	

* TRY- CONVERTANTS PER PLATE

** TA-1535 EMS 10 UL/PLATE
TA-1537 DM 10 UG/PLATE
TA-1538 NF 10 UG/PLATE
TA-98 NF 10 UG/PLATE
TA-100 EMS 10 UL/PLATE
DA EMS 10 UL/PLATE
SOLVENT DMSO 50 UL/PLATE

*** TA-1535 ANTH 2.5 UG/PLATE
TA-1537 ANTH 2.5 UG/PLATE
TA-1538 ANTH 2.5 UG/PLATE
TA-98 ANTH 2.5 UG/PLATE
TA-100 ANTH 2.5 UG/PLATE
DA DMNA 100 MICROMOLES/PLATE
SOLVENT DMSO 50 UL/PLATE

* THERE IS NO KNOWN POSITIVE CONTROL COMPOUND THAT WORKS WITH THIS STRAIN IN THE ACTIVATION PLATE ASSAYS.

PROTOCOL

1. PURPOSE

The purpose of this study was to evaluate the test material for genetic activity in a microbial assay with and without the addition of mammalian metabolic activation preparations.

2. MATERIALS

A. Indicator Microorganisms

A description of strain verification is given in Standard Operating Procedure on page 10.

Salmonella typhimurium	TA-1535
	TA-1537
	TA-1538
	TA-98
	TA-100
Saccharomyces cerevisiae	D4

B. Activation System

1. Reaction mixture

Component	Final Concentration/ml
TPN (sodium salt)	4 µmol
Glucose-6-phosphate	5 µmol
Sodium phosphate (dibasic)	100 µmol
$MgCl_2$	8 µmol
KCl	33 µmol
Homogenate S9 fraction	0.1 ± .05 ml

2. S9 homogenate

A 9,000 x g supernatant was prepared from Sprague-Dawley adult male rat liver induced by Aroclor 1254 five days prior to kill according to the procedure of Ames et al. (1975). S9 samples were coded by lot number and assayed for milligrams protein per milliliter and relative P448/P450 activity by methods described in LBI Technical Data on Rat Liver S9 Product.

2. MATERIALS (Continued)

C. Positive Control Chemicals

The chemicals used for positive controls in the nonactivation and activation assays are given in Table 1 of Section V. Results.

D. Solvent

Either deionized water or dimethylsulfoxide (DMSO) was used to prepare stock solutions of solid materials. All dilutions of test materials were made in either deionized water or DMSO. The solvent employed and its concentration are recorded in Table 1 of Section V. Results.

3. EXPERIMENTAL DESIGN

A. Plate Test (Agar Incorporation)*

Approximately 10^8 cells from an overnight culture of each indicator strain were added to separate test tubes containing 2.0 ml molten agar supplemented with biotin and a trace of histidine. For nonactivation tests, at least 4 dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests, at least 4 dose levels of the test chemical were added to the appropriate tubes with cells. Just prior to pouring, an aliquot of reaction mixture (0.5 ml containing the 9,000 x g liver homogenate) was added to each of the activation overlay tubes, which were then mixed, and the contents poured over the surface of a minimal agar plate and allowed to solidify. The plates were incubated for 48 hr at 37°C and scored for the number of colonies growing on each plate. D4 yeast plates were incubated at 30°C (nonactivation) and 37°C (activation) for 3-5 days and then scored. The concentrations of all chemicals are given in Table 1 of Section V. Results. Positive and solvent controls using both directly active positive chemicals and those that require metabolic activation were run with each assay.

---

* Certain classes of chemicals known to be mutagens and carcinogens do not produce detectable responses using the standard Ames agar incorporation method. Some dialkyl nitrosamines and certain substituted hydrazines are mutagenic in suspension assays, but not in the plate assay. Chemicals of these classes should be screened in a suspension assay.

3. EXPERIMENTAL DESIGN (Continued)

B. Recording and Presenting Data

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were analyzed in a computer program and reported on a printout. The results are presented as revertants (or convertants for D4) per plate for each indicator strain employed in the assay. The positive and solvent controls are provided as reference points. Other relevant data are provided on the computer printout.

4. EVALUATION CRITERIA

Plate test data consist of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test chemical and the cells are incubated in the overlay for 2 days, and a few cell divisions occur during the incubation period, the test is semiquantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test:

- The small number of cell divisions permits potential mutagens to act on replicating DNA, which is often more sensitive than nonreplicating DNA.
- The combined incubation of the compound and the cells in the overlay permits constant exposure of the indicator cells for 2 days.

A. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test chemical, the surviving population on the treatment plates is essentially the same as that on the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol normally employs several doses ranging over 2 or 3 log concentrations, the highest of these doses being selected to show slight toxicity as determined by subjective criteria.

B. Dose-Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. A factor that might modify dose-response results for a mutagen would be the

4. EVALUATION CRITERIA (Continued)

B. Dose-Response Phenomena

selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test chemical may kill any mutants that are induced, and the compound will not appear to be mutagenic.

C. Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct-acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assays. Negative controls consist of the test compound solvent in the overlay agar together with the other essential components. The negative control plate for each strain gives a reference point to which the test data are compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.

D. Evaluation Criteria for Ames Assay

Because the procedures used to evaluate the mutagenicity of the test chemical are semiquantitative, the criteria used to determine positive effects are inherently subjective and are based primarily on a historical data base. Most data sets are evaluated using the following criteria:

1. Strains TA-1535, TA-1537, and TA-1538

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the lowest increase equal to twice the solvent control value is considered to be mutagenic.

2. Strains TA-98, TA-100, and D4

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value for TA-100 and 2-3 times the solvent control value for strains TA-98 and D4 is considered to be mutagenic. For these strains, the dose-response increase should start at approximately the solvent control value.

4. EVALUATION CRITERIA (Continued)

D. Evaluation Criteria for Ames Assay

3. Pattern

Because TA-1535 and TA-100 are both derived from the same parental strain (G-46) and because TA-1538 and TA-98 are both derived from the same parental strain (D3052), there is a built-in redundancy in the microbial assay. In general the two strains of a set respond to the same mutagen and such a pattern is sought. It is also anticipated that if a given strain, e.g., TA-1537, responds to a mutagen in nonactivation tests, it will generally do so in activation tests (The converse of this relationship is not expected.). While similar response patterns are not required for all mutagens, they can be used to enhance the reliability of an evaluation decision.

4. Reproducibility

If a chemical produces a response in a single test that cannot be reproduced in one or more additional runs, the initial positive test data lose significance.

The preceding criteria are not absolute, and other extenuating factors may enter into a final evaluation decision. However, these criteria are applied to the majority of situations and are presented to aid those individuals not familiar with this procedure. As the data base is increased, the criteria for evaluation can be more firmly established.

E. Relationship between Mutagenicity and Carcinogenicity

It must be emphasized that the Ames Salmonella/Microsome Plate Test is not a definitive test for chemical carcinogens. It is recognized, however, that correlative and functional relationships have been demonstrated between these two endpoints. The results of comparative tests on 300 chemicals by McCann et al. (1975) show an extremely good correlation between results of microbial mutagenesis tests and in vivo rodent carcinogenesis assays.

All evaluations and interpretation of the data presented in this report are based only on the demonstration, or lack, of mutagenic activity.

REFERENCES

Ames, B.N., McCann, J. and Yamasaki, E. (1975). Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutation Res. 31, 347-364.

McCann, J., Choi, E., Yamasaki, E. and Ames, B.N. (1975). Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. Proc. Nat. Acad. Sci. 72, 5135-5139.

5. STANDARD OPERATING PROCEDURE

All data will be entered in ink (no pencil).

All changes or corrections in entries will be made with a single line through the change, and an explanation for the change must be written.

All calculations (weights, dilutions, dose calculations, etc.) will be shown on data records.

All data entries will be dated and initialed.

All laboratory operations will be written out in standard protocol manuals. These manuals will be present in each laboratory area.

Deviations from any established protocol will be described and justified.

Data will be stored in bound form (notebooks or binders). These bound data books will be reviewed by the appropriate Section Heads.

Chemicals submitted for testing will have date of receipt and initials of entering person.

Lot numbers for all reference mutagens, solvent, or other materials used in assays will be recorded.

Animal orders, receipts, and identification will be recorded and maintained such that each animal can be traced to the supplier and shipment. All animals on study will be properly identified.

A copy of the final report plus all raw data and support documents will be permanently stored in the archival system of Litton Bionetics, Inc.

Current curricula vitae and job descriptions will be maintained on all personnel involved in the study.

Salmonella strains will be routinely checked for the his, uvrB, rfa, and pKM 101 phenotypes. Only appropriately screened stock cultures will be used in chemical evaluations.

PULLIN, T.G. 1977
BALLANTYNE, M. 2000
BOOGARD, P.J. et al. 2000

EcoToxConsulting
Dr. Hans-Jörg Weideli
Weissenbachlistrasse 4
CH-3775 Lenk

Lenk, October 04 2006

Report on the Classification and Labelling Meeting in Arona (Italy) on October 4, 2006

o-CGE was tabled for 16:00 on October 4th, 2006.
As a frist speaker, the Italian representative (Dottoressa P. di Prospero) brought forward arguments to abandon the classification and labelling for mutagenicity category 3.Germany followed with similar arguments, but Finland, Sweden and Norway argued for not changing the classification and labelling.
In fact, The Netherlands were the ones bringing forward very convincing arguments: they claimed that their experts have come to the conclusion that Transgenic Rodent Assays are state-of-the-art, and while European authorities have asked industry to perform such tests, they also have to accept such tests. The Transgenic Mouse assay with o-CGE was judged by the Dutch Experts as being valid and clearly negative !
The Nordic Countries however wanted to question the validity of such assays and pleaded for maintaining the classification.
That is when I asked for the word; I explained them that at the end of the last meeting it was decided to come to a conclusion on whether Transgenic Rodent Assays should be accepted for classification and labelling , and if yes, whether this particular assay was good evidence for Non-mutagenicity in-vivo.
The arguments of the Dutch Authorities swichted the opinion towards abandoning the the mutagenicity classification.

It was finally decided that

Transgenic Rodent Assays are acceptable for classification & labelling

and

The classification & labelling concerning the mutagenicity category 3 is removed !!

At the end of this session the chairwomen was proud that they took a very unusual decision (declassification of a substance).

Votes:

Italy.	NO classification
Germany:	NO classification
Netherlands	NO classification
UK:	partly in favour of no classification
France:	no comment
Belgium:	no comment
Finland:	weakly for keeping the classification (no strong arguments)
Sweden:	weakly for keeping the classification (no strong arguments)
Norway:	weakly for keeping the classification (no strong arguments)

Alll other country representatives remained silent

Hans-Jörg Weideli

PlasticsEurope
Association of Plastics Manufacturers

Epoxy Resins Group
Dr. Hans-Jörg Weideli June 2006

Re-evaluation of classification & labelling of o-cresyl glycidylether (o-CGE)

Background

According to the 25th adaptation to Technical progress (Commission Directive 98/98/EC of December 1998), o-CGE (CAS-no. 2210-79-9) has to be classified and labelled as follows:

Xn, N, R 38-40-43 and R 51/53 (Mutagen Cat. 3).

It has to be mentioned that the general public is not exposed to o-CGE, as it is fully reacted in all items in which it is used as reactive diluent.

Classification of o-CGE has been performed in 1999, shortly before the Transgenic Mutation Assay in Muta™Mice of o-CGE has been finalized by industry. Only a summary of the study was available for the classification and labelling group, and the metabolism and skin penetration studies were not yet available.

The classification as Mutagen Category 3 applies to all three isomers of cresyl glycidylether, but is based upon studies performed exclusively with o-CGE. Some studies are summarized in Gardiner, T.H. et al. (1992) and can be described as follows:

o-CGE is positive in the Salmonella typhimurium reverse mutation assay (Ames test) in strains TA 1535 and TA 100 in the absence, and in strain TA 1535 in the presence of rat liver metabolic enzymes. Since no mutagenic effect was observed in strain TA 98, it is concluded that o-CGE itself is active in base-pair mutations as a direct acting mutagen (Canter, D.A. et al. 1986, Neau, S.A. et al., 1982). These data are in good agreement with those for other glycidyl ethers.
In an unscheduled DNA-repair assay in isolated human lymphocytes, o-CGE induced a significant increase in unscheduled DNA-synthesis at 10 and 100 ppm, but caused a significant reduction at 1000 ppm due to its cytotoxic effects (Pullin, 1977).
In a "in-vivo"/"in-vitro" mutagenicity assay, female B6D2F1 and ICR mice were dosed by gavage with 125 mg/kg body weight of o-CGE in corn oil for four consecutive days. Urine was collected shortly thereafter and Salmonella typhimurium strain TA 1535 (with and without supplementation of β-glucuronidase) was exposed to urine samples. No mutagenic activity was detected in the urine of B6D2F1 mice neither in the presence or absence of β-glucuronidase, but urine of ICR mice caused an increase in mutations in the presence of β-glucuronidase Pullin, 1977). The possible difference in metabolism by the two mouse strains remains to be explained.

In a dominant-lethal assay o-CGE was administered dermally to B6D2F1 mice at doses of 1500 mg/kg body weight, three times per week for 8 consecutive weeks. A significant decrease in the mean number of implants was recorded suggesting pre-implantation loss; however, there was no increase in post-implantation loss. This result was interpreted to show general toxic effects in the dams rather than a genotoxic effect causing interruption of the normal early development (Pullin, 1977).
Haemoglobin adducts were measured in male C3H/Hej-mice 24 hours after a dose injected intraperitoneally (4 mg/mouse ,= approx. 160 mg/kg body weight). The Haemoglobin Binding Index (HBI) was found to be 1.3, which is in the range of other glycidyl ethers, but relatively low compared to known carcinogens (Perez, H.L. et al, 1997).

The data available at the time for classification and labelling clearly indicate that o-cresyl glycidyl ether is a direct acting mutagen "in-vitro", but little evidence was available for its possible "in-vivo" mutagenicity, based solely on the haemoglobin-adduct data and the dominant-lethal assay described above (see also Appendix "o-Cresyl Glycidyl Ether, 2000").

New Evidence for the lack of "in-vivo" mutagenicity

(All recent studies have been distributed on the Agenda of the Classification & Labeling Meeting in Arona, 2006).

Since 1999, a series of metabolism and transdermal penetration studies have been performed by industry in order to evaluate the potential hazard of o-CGE with respect to worker exposure and worker protection. The studies were conducted with ^{14}C-labeled o-CGE, which was synthesized according to standard procedures (van Elburg, P.A. et al., 2000).

Percutaneous penetration and metabolism of o-CGE was studied *in-vitro* in fresh preparations of skin of mouse, rat and human (Boogaard, P.J. et al., 2000a). o-CGE was applied to skin preparations at a concentration of 5.0 µmol/cm^2 as ^{14}C-labeled product, and percutaneous penetration was measured over a 24-hour period, showing that a plateau was reached at about 15-20 hours with all three skin types. Penetration was highest with mouse skin followed by rat and finally by human skin, whereby about 22% of the applied dose penetrated mouse skin, and 10 % penetrated human skin. Over time the hydrolysis of o-CGE during skin penetration was inhibited, and increasing amounts of unchanged product passed the skin with increasing time, thus exposing the remaining part of the body to the potential mutagenic activity of the epoxide moiety of the molecule.

In a further study (Boogaard, P.J. et a., 2000b) the metabolic behaviour of ^{14}C-labeled o-CGE was studied using lung and liver cytosolic and microsomal fractions from mouse, rat and human. Both, hydrolysis by epoxide hydrolases and conjugation with glutathione by glutathione-S-transferase (GST) were investigated. o-CGE was both a good substrate for GST as well as for epoxide hydrolases, whereby conjugation with glutathione was more efficient in liver as compared to lung, and highest in mouse liver cytosol, while human lung was more efficient than rat and mouse lung with respect to conjugation. Hydrolysis by epoxide hydrolases was most efficient by human microsomal enzymes, followed by rat and mouse microsomal enzymes. Cytosolic activity was less pronounced, but human cytosolic fractions were more efficient than those of rats and mice. Lung cytosolic fractions were the least efficient, but again human sub-cellular fractions were the most efficient.

Taken the results of these biochemical studies together, it can be concluded that among humans, rats and mice, the mouse is the most sensitive species, since mouse detoxification of

o-CGE by GST conjugation and epoxide hydrolase inactivation is the least efficient, and, therefore, o-CGE is likely to show the most severe effects in this animal species.

These new metabolic studies triggered the conducting of an "*in-vivo*" mutagenicity study in transgenic mice (Muta™Mouse), allowing experimental work to be done on the potential mutagenic activity in different mouse target tissues, such as skin (site of first contact), bone-marrow and liver. Transgenic rodent systems have been described and investigated by several groups, and the overall conclusion was favourable, e.g. the system provided data in line with results generated by test systems based on OECD guidelines (Brooks, T.M. et al. 1995; Dean, S.W. & Myhr, B. 1994; Miyata, Y. et al. 1998; Davies, R. et al. 1999; Lefevre, P.A. et al. 1997; van Kreijl, C.F. & van Steeg, H. 1998).

o-CGE readily penetrates the skin in mice without being extensively metabolised; metabolism occurs mainly in the liver as deduced from the above studies. Therefore, the dermal route of exposure in the *in-vivo* mutagenicity assay is justified, both from a biological/toxicological point of view as well as from a worker hygiene point of view.

In the transgenic Muta™Mice the bacterial LacZ gene complex is stably integrated into each cell of the animals by gene transfer technology. The advantage of using the transgenic Muta™Mice system is the possibility to analyse any organ and tissue of the mouse to perform a gene mutation assay *in-vivo*, while any other test system would allow only one tissue for chromosomal aberration or UDS analysis. Unlike the only gene mutation test *in-vivo* available in mice, the mouse spot test, the transgenic Muta™Mice system requires only a limited number of animals, and is of relatively short duration. The stably integrated bacterial genes can easily be extracted and analysed for gene mutations. Furthermore, an extension of the assay would even allow different types of gene mutations such as base-changes, insertions or deletions to be distinguished. These genes can be quantitatively isolated at any time after exposure, and from any tissue of the mice. Furthermore, these genes can then be integrated into plasmid vectors, multiplied in bacteria, and the presence or absence of LacZ gene expression can be monitored, indicating the presence or absence of gene mutations (Ballantyne, M., 2000).

In a dose – range experiment the dose of 500 mg/kg body weight was found to be the maximum tolerated dose, whilst doses of 1000 and 2000 mg/kg body weight caused severe health effects in a number of treated animals. In the main experiment only one dose level of 500 mg/kg body weight was analysed (including a control group); in this group 5 male mice were treated on five consecutive days. Animals were sacrificed on day 12 and 33, allowing an expression time of 7 and 28 days following the last application. The expression time is the time required to fully stabilize the induced mutations in the LacZ gene system, and is important for the sensitivity of the assay system. A group of 5 male mice treated dermally for 5 consecutive days with 0.25 mg/kg benzo[a]pyrene served as positive control; these animals were sacrificed on day 12 after the first application. After necropsy the bone-marrow, liver and skin were dissected, flash-frozen and stored in liquid nitrogen until DNA extraction. The isolated DNA was cut with appropriate restriction enzymes, introduced into bacteriophage lambda, and competent Escherichia coli bacteria were transfected with the phages, and plated on soft agar in the presence of phenylgalactose. Plates were scored for clear plaques. Mutation frequencies (MF) were calculated based on scoring in excess of 200'000 plaques per tissue as mean MF per 10^6 plaques.

While all the treated tissue samples were within the control background MF, the positive control showed a MF about 7 times higher than the control, indicating the sensitivity of the test system. The statistically significant increase of the mutation frequency in bone-arrow

after the short expression time (but not the long one) was considered not to be biologically significant. In addition, there was no significant difference between the two expression times. From the data it can be concluded that o-CGE did not induce mutations in the LacZ gene system introduced stably into the mouse genome, neither at the site of first contact (skin), nor in the liver (major metabolic organ) and the bone-marrow.

Bone-marrow is known to show a high variability of the mutation frequency between individual animals and during the first 6 months of life in mice. Slightly increased mtation frequencies are often related to clonal expansion of mutant cells as compared to normal cells. For a positive result the mutation frequency should be elevated at least by a factor of two (Lambert et al., 2005). As the mutation frequency in bone-marrow is extremely low as compared to other organs, variability among different animals is likely to be higher than in other organs.

Interpretation and Conclusions

Based on the EU Directive 1999/45/EC, Article 4, and EU Directive 67/548/EEC, Article 4, Annex VI section 4.2.2.3 [mutagens category 3]), *"substances showing positive results only in one or more in-vitro mutagenicity assays should normally not be classified. Their further investigation using in-vivo assays, however is strongly indicated. In exceptional cases, e.g. for substances showing pronounced responses in several in-vitro assays, for which no relevant in-vivo data are available, and which shows resemblance to known mutagens/carcinogens, classification in category 3 should be considered."*

With the attached mutagenicity and pharmaco-kinetic assays, we demonstrate that , following the criteria of the Directives, no classification and labelling for o-CGE is evident.

This *in-vivo* study is accepted in the scientific community to be superior to any other *in-vivo* mutagenicity study presently available. Although it has no regulatory status yet, the majority of toxicologists are very enthusiastic about this type of mutagenicity study, which opens doors to new investigations almost impossible before. The validation of the test system has reached a level, which allows substances to be tested with adequate security and acceptance (Lambert et al., 2005).

Studies with transgenic Muta™Mice have been performed in the mean-time with a variety of chemicals including several epoxy compound among them BADGE (bisphenol-A diglycidyl ether) and NOGE (epoxy novolac resins). Both epoxides showed no *in-vivo* mutagenic potential when mice were exposed orally to the compounds, which is in line with the negative carcinogenicity study of BADGE. Both studies were accepted for risk assessment (indirect food contact) by EFSA. The UK Committee for Carcinogens (CoC) and the Committee for Mutagens (CoM) as well as the IPCS expressed their interest in using the Transgenic Rodent Assay System for quantitative risk assessment (QRA) as expressed in their respective publications (Committee on Mutagenicity, 2000). The Canadian Authorities have expressed their opinion about the usefulness and advantages for risk assessments of transgenic rodent system in a review article (Lambert et al., 2005)

The study with transgenic Muta™Mice with o-CGE showed that the compound has no detectable mutagenic potential in-vivo, although significant *in-vitro* mutagenicity has been observed in bacterial and mammalian cell culture experiments. In this *in-vivo* test the

mutagenic potential of o-CGE has been evaluated in mouse skin, the site of first contact, in bone-marrow, a tissue with high mitotic activity, and in liver, the site of the most extensive epoxide hydrolase activity. In contrast to cell culture assays, the transgenic Muta™Mice allow evaluation of mutagenic effects in cells which are fully metabolising unlike cell culture cells which show in general a strongly reduced pattern of enzyme activities as compared to cells *in-situ*. This situation is closer to real life situations and therefore should over-rule all previous studies performed with inferior test systems.

Based on these considerations, we suggest to re-evaluate the classification and labelling of o-CGE (CAS-numbers: 2210-79-3, EC-number: 218-645-3), and to abandon the classification as Mutagen cat. 3, to yield a newclassifiaction and labelling as

Xi, N; R 38-43, 51/53

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