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Document Title		INITIAL SUBMISSION: LETTER FROM METHACRYLATE PRDCRS ASSN INC TO USEPA RE SUMMARIES OF METHACRYLATE TOXICITY STUDIES CONDUCTED IN JAPAN, WITH ATTACHMENTS AND DATED 8/2/1999			
Chemical Category		BUTYL METHACRYLATE, TERT-BUTYL METHACRYLATE, GLYCIDYL			

A 03

METHACRYLATE PRODUCERS ASSOCIATION, INC.

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Office: (202) 637-9040 • Facsimile: (202) 637-9178

8EHQ-0899-14519

August 2, 1999

via messenger

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Attn: TSCA Section 8(e) Coordinator
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
401 M Street, SW, Room G-099
Washington, DC 20460

MR 24962

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- Re: Notice in Accordance with TSCA Section 8(e) --
- Butyl Methacrylate (CAS No. 97-88-1)
 - tert-Butyl Methacrylate (CAS No. 585-07-9)
 - Glycidyl Methacrylate (CAS No. 106-91-2)
 - Dimethylaminoethyl Acrylate (CAS No. 2439-35-2)
 - 2-(Dimethylamino)ethyl Methacrylate (CAS No. 2867-47-2)
 - 2-(Diethylamino)ethyl Methacrylate (CAS No. 105-16-8)
 - 2-Ethylhexyl Methacrylate (CAS No. 688-84-6)
 - 2-Hydroxypropyl Methacrylate (CAS No. 923-26-2)
 - 2-Hydroxyethyl Methacrylate (CAS No. 868-77-9)

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9:10-98-14519

Dear Sir/Madam:

The Methacrylate Producers Association, Inc. (MPA) is submitting results from several studies conducted in Japan on the above compounds. These data are likely to be submitted under the ICCA Program. Although it is not clear if these data are reportable, MPA is making this submission in accordance with TSCA Section 8(e). Attached to this submission are summary reports on each compound.

This submission is being made on behalf of the MPA member companies: CYRO Industries, Elf Atochem N.A., Inc., ICI Acrylics, Inc., and Rohm and Haas Company. If you have any questions about this submission, please contact me at (202) 637-9040.

Contain NO CBI

Sincerely,

Elizabeth K. Hunt
Executive Director

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88990000238

Ministry of Health and Welfare: Japan, *Toxicity Testing Reports of Environmental Chemicals* 6, 77-100 (1998)

Butyl methacrylate

[CAS No. 97-88-1]

Molecular formula: C₈H₁₄O₂

Molecular weight: 142.20

ABSTRACT

Butyl methacrylate was studied for oral toxicity in rats in an OECD combined repeat dose and reproductive/developmental toxicity screening test at doses of 0, 30, 100, 300 and 1000 mg/kg/day.

With regard to repeat dose toxicity in males, weight gain depression and a decrease in food consumption were observed at a dose of 1000 mg/kg. Urinary examination revealed increases in ketone bodies and occult blood, and hematological and blood chemical examinations showed increases in prothrombin time and urea nitrogen at a dose of 1000 mg/kg. Absolute and relative weights of the spleen were decreased at a dose of 100 mg/kg or more, and relative kidney weights were increased at a dose of 1000 mg/kg. Histopathological examination revealed atrophy of the splenic red pulp at doses of 100 mg/kg or more. The kidney showed no histopathological abnormalities attributable to the test substance. In females, there were weight gain depression and a decrease in food consumption at a dose of 1000 mg/kg. Atrophy of the red pulp in the spleen was also observed histopathologically at a dose of 1000 mg/kg. The NOELs for repeat dose toxicity are considered to be 30 mg/kg/day for males and 300 mg/kg/day for females. In terms of reproductive and developmental toxicity, there were decreases in numbers of corpora lutea and implantations in the parental females. The test substance showed no effects on any reproductive parameters of the parental males or developmental parameters of the offspring. The NOELs for reproductive and developmental toxicity are considered to be 1000 mg/kg/day for the parental males and offspring, and 300 mg/kg/day for the parental females.

Butyl methacrylate was not mutagenic in *Salmonella typhimurium* TA100, TA1535, TA98, TA1537 and *Escherichia coli* WP2 *uvrA*, with or without an exogenous metabolic activation system.

Butyl methacrylate did not induce structural chromosomal aberrations or polyploidy in CHL cells, with or without an exogenous metabolic activation system.

SUMMARIZED DATA FROM THE STUDIES

1. Repeat Dose and Reproductive/Developmental Toxicity1)

Purity: 99.6 %

Test species/strain: Rat/Crj: CD (SD)

Test method: OECD Combined Repeat Dose and Reproductive/ Developmental Toxicity Screening Test

Route: Oral (gavage)

Doses: 0(vehicle), 30, 100, 300, 1000 mg/kg/day

Number of animals/group: Males, 10; females, 10/group

Vehicle: Sesame oil

Administration period: Males, 44 days

Females, from 14 days before mating to day 3 of lactation

Terminal kill: Males, 45 days

Females, day 4 of lactation

GLP: Yes

Test results:

<Repeat Dose Toxicity>

In males, there were weight gain depression and a decrease in food consumption at a dose of 1000 mg/kg. Urinary examination revealed increases in ketone bodies and occult blood, and

hematological and blood chemical examinations showed increases in prothrombin time and urea nitrogen at a dose of 1000 mg/kg. Absolute and relative weights of the spleen were decreased at a dose of 100 mg/kg or more, and relative kidney weights were increased at a dose of 1000 mg/kg. Histopathological examination revealed atrophy of the splenic red pulp at doses of 100 mg/kg or more. The kidneys showed no histopathological abnormalities attributable to the test substance.

In females, there were weight gain depression and a decrease in food consumption at a dose of 1000 mg/kg. Atrophy of the red pulp in the spleen was also observed histopathologically at a dose of 1000 mg/kg.

The NOELs for repeat dose toxicity are considered to be 30 mg/kg/day for males and 300 mg/kg/day for females.

<Reproductive and developmental toxicity>

There were decreases in numbers of corpora lutea and implantations in the parental females. The test substance showed no effects on any reproductive parameters of the parental males or developmental parameters of the offspring.

The NOELs for reproductive and developmental toxicities are considered to be 1000 mg/kg/day for the parental males and offspring, and 300 mg/kg/day for the parental females.

2. Genetic Toxicity

2-1. Bacterial test(2)

Purity: 99.6 %

Test species/strains: *Salmonella typhimurium* TA100, TA1535, TA98, TA1537, *Escherichia coli* WP2 uvrA

Test methods: Guidelines for Screening Mutagenicity Testing of Chemicals (Japan) and OECD Guidelines No. 471 and 472

Procedures: Pre-incubation method

Solvent: DMSO

Positive controls: -S9 mix; 2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide (TA100, TA98 and WP2 uvrA), Sodium azide (TA1535), 9-Aminoacridine hydrochloride (TA1537)
+S9 mix, 2-aminoanthracene (all strains)

Doses: -S9 mix: 9.77, 19.5, 39.1, 78.1, 156, 313 µg/plate (TA100, TA1535, TA98 and TA1537);
9.77, 19.5, 39.1, 78.1, 156, 313 and 625 µg/plate (WP2 uvrA)

+S9 mix: 9.77, 19.5, 39.1, 78.1, 156, 313 and 625 µg/plate (TA100); 19.5, 39.1, 78.1, 156, 313, 625 and 1250 µg/plate (TA1535, TA1537 and WP2 uvrA); 9.77, 19.5, 39.1, 78.1, 156 and 313 µg/plate (TA98)

S9: Rat liver, induced with phenobarbital and 5,6-benzoflavone

Plates/test: 3

Number of replicates: 2

GLP: Yes

Test results:

This chemical did not induce gene mutations in the *S. typhimurium* and *E. coli* strains.

Toxicity was observed at a concentration of 156 µg/plate in the five strains without an S9 mix, and at 313 µg/plate or greater (TA100, TA1535, TA98, TA1537) and 625 µg/plate or greater (WP2 uvrA) with an S9 mix.

Genetic effects:

Salmonella typhimurium TA100, TA1535, TA98, TA1537

	+	?	-
Without metabolic activation	[]	[]	[*]
With metabolic activation	[]	[]	[*]

Escherichia coli WP2 uvrA

	+	?	-
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Without metabolic activation	[]	[]	[*]
With metabolic activation	[]	[]	[*]

2-2. Non-bacterial in vitro test (chromosomal aberration test)2)

Efficiency: 99.6%

Type of cell used: Chinese hamster lung (CHL) cells

Test method: Guidelines for Screening Mutagenicity Testing of Chemicals (Japan) and OECD Guideline No. 473

Solvent: DMSO

Positive controls: -S9 mix, Mitomycin C

+S9 mix, Cyclophosphamide

Doses: -S9 mix (continuous exposure): 0, 178, 355, 710, 1420 µg/mL

-S9 mix (short-term exposure): 0, 178, 355, 710, 1420 µg/mL

+S9 mix (short-term exposure): 0, 355, 710, 1420 µg/mL

S-9: Rat liver, induced with phenobarbital and 5,6-benzoflavone

Plates/test: 2

GLP: Yes

Test results:

This chemical did not induce structural chromosomal aberrations in the absence or presence of an exogenous metabolic activation system.

Genotoxic effects:	clastogenicity			polyploidy		
	+	?	-	+	?	-
Without metabolic activation:	[]	[]	[*]	[]	[]	[*]
With metabolic activation:	[]	[]	[*]	[]	[]	[*]

- 1) The tests were performed by the Research Institute for Animal Science in Biochemistry and Toxicology, 3-7-11 Hashimoto-dai, Sagami-hara-shi, Kanagawa 229-1132, Japan. Tel +81-42-762-2775 Fax +81-42-762-7979
- 2) The tests were performed by the Biosafety Research Center, Foods, Drugs and Pesticides (Anpyo Center), Japan, 582-2 Shioshinden Arahama, Fukuda-cho, Iwata-gun, Shizuoka, 437-1413, Japan. Tel +81-538-58-1266 Fax +81-538-58-1393

Ministry of Health and Welfare: Japan, *Toxicity Testing Reports of Environmental Chemicals 4*, 485-507 (1996)

tert-Butyl methacrylate

[CAS No. 585-07-9]

Molecular formula: C₈H₁₄O₂ Molecular weight: 142.20

ABSTRACT

tert-Butyl methacrylate was studied for oral toxicity in rats in a 28-day repeat dose toxicity test at doses of 0, 20, 100 and 500 mg/kg/day.

Transient salivation after administration was observed in the 500 mg/kg/day group. Blood chemical examination showed increases in total cholesterol, total protein, and albumin, and a decrease in alkaline phosphatase in the 100 and 500 mg/kg/day groups. Urinalysis showed increases in protein, occult blood, bilirubin, erythrocytes, and epithelial cells in the 100 and 500 mg/kg/day groups. Absolute and relative weights of the liver and kidneys increased in the 500 mg/kg/day group and relative liver weights were increased in the 100 mg/kg/day group. Histopathological examination showed centrilobular hypertrophy of hepatocytes, an increase in hyaline droplets in the proximal renal tubules and basophilic change of renal tubules in the 100 and 500 mg/kg/day groups. The NOEL for the repeat dose toxicity is considered to be 20 mg/kg/day for both sexes.

tert-Butyl methacrylate was not mutagenic to *Salmonella typhimurium*, TA100, TA1535, TA98, TA1537 and *Escherichia coli* WP2 uvrA. In the absence of an exogenous metabolic activation system, this chemical induced structural chromosomal aberrations in CHL/IU cells. Polyploidy was not induced under the conditions of the present study.

SUMMARIZED DATA FROM THE STUDIES

1. Repeat Dose Oral Toxicity 1)

Purity: 99.8 %

Test species/strain: Rat/Crj:CD (SD)

Test method: Guidelines for 28-Day Repeat Dose Toxicity Testing for Chemicals (Japan)

Route: Oral (gavage)

Doses: 0 (vehicle), 20, 100, 500 mg/kg

Number of animals/group: Males, 6; females, 6

Vehicle: 0.5% Sodium Carboxymethyl Cellulose solution containing 0.1% Tween 80

Administration period: Males and females, 28 days

Terminal kill: Days 29 or 43

GLP: Yes

Test results:

There were no deaths throughout the course of the study. Transient salivation after administration was observed in both sexes given 500 mg/kg/day. The body weights and food consumption revealed no differences between the control and treated groups. There were no observed effects of the test substance on hematological findings. Blood chemical examination showed increases in total cholesterol and total protein in both sexes given 100 and 500 mg/kg/day, an increase in albumin in females given 100 mg/kg/day and both sexes given 500 mg/kg/day, and a decrease in alkaline phosphatase in males given 100 mg/kg/day and both sexes given 500 mg/kg/day. Urinalysis showed an increase in protein in both sexes given 500 mg/kg/day, an increase in occult blood in males given 100 and 500 mg/kg/day and an increase in bilirubin in males given 500 mg/kg/day. In addition, microscopic examination of urinary sediment revealed an increase in erythrocytes in males given 500 mg/kg/day and an increase in epithelial cells in females given 500 mg/kg/day. Absolute and relative liver and kidneys weight were increased in both sexes

given 500 mg/kg/day, and relative weight of the liver increased in males given 100 mg/kg/day. Necropsy revealed hypertrophy of the liver in three males and five females given 500 mg/kg/day. Histopathological examination showed centrilobular hypertrophy of hepatocytes in four males given 100 mg/kg/day and all animals of both sexes given 500 mg/kg/day. Increase in the amounts of hyaline droplets of proximal renal tubules was observed in two males given 100 mg/kg/day and four males given 500 mg/kg/day. Basophilic change of renal tubules was observed in one male given 100 mg/kg/day and three males given 500 mg/kg/day. These changes disappeared or tended to recover during the recovery period.

The NOEL for the repeat dose toxicity is considered to be 20 mg/kg/day for both sexes.

2. Genetic Toxicity

2-1. Bacterial test 2)

Purity: 99.8%

Test species/strains: *Salmonella typhimurium* TA100, TA1535, TA98, TA1537, *Escherichia coli* WP2 uvrA

Test methods: OECD guideline (No. 471, 472) and Guidelines for Screening Mutagenicity Testing of Chemicals (Japan)

Procedures: Modified pre-incubation method for volatile substances

Solvent: Acetone

Positive controls: -S9 mix, AF-2 (TA100, TA98), sodium azide (TA1535), ENNG (WP2 uvrA) and 9-aminoacridine (TA1537)
+S9 mix, 2-aminoanthracene (all strains)

Doses: -S9 mix: 9.77, 19.5, 39.1, 78.1, 156, 313 µg/plate (TA100) 19.5, 39.1, 78.1, 156, 313, 625 µg/plate (TA1535, TA98, TA1537, WP2 uvrA)

+S9 mix: 9.77, 19.5, 39.1, 78.1, 156, 313 µg/plate (TA100) 19.5, 39.1, 78.1, 156, 313, 625 µg/plate (TA1535, TA98, TA1537, WP2 uvrA)

S9: Rat liver, induced with phenobarbital and 5,6-benzoflavone

Plates/test: 3

Number of replicates: 2

GLP: Yes

Test results:

This chemical did not induce gene mutations in the *S. typhimurium* and *E. coli* strains. Toxicity was observed at a concentration of 313 µg/plate, with or without metabolic activation.

Genetic effects:

Salmonella typhimurium TA100, TA1535, TA98, TA1537

	+	?	-
Without metabolic activation	[]	[]	[*]
With metabolic activation	[]	[]	[*]

Escherichia coli WP2 uvrA

	+	?	-
Without metabolic activation	[]	[]	[*]
With metabolic activation	[]	[]	[*]

2-2. Non-bacterial in vitro test (chromosomal aberration test) 2)

Purity: 99.8%

Type of cell used: Chinese hamster CHL/IU cells

Test method: OECD guideline (No. 473) and Guidelines for Screening Mutagenicity Testing of Chemicals (Japan)

Solvent: Acetone

Positive controls: -S9 mix, Mitomycin C

+S9 mix, Benzo[a]pyrene

Doses: -S9 mix (24 h treatment): 0, 100, 200, 400 µg/ml
 -S9 mix (48 h treatment): 0, 50, 100, 200 µg/ml
 -S9 mix (6 h pulse treatment): 0, 175, 350, 700 µg/ml
 +S9 mix (6 h pulse treatment): 0, 188, 375, 750 µg/ml
 S-9: Rat liver, induced with phenobarbital and 5,6-benzoflavone
 Plates/test: 2
 GLP: Yes

Test results:

This chemical induced structural chromosomal aberrations in the presence of an exogenous metabolic activation system.

Lowest concentration producing cytogenetic effects in vitro:

Without metabolic activation (24 h treatment): 400 µg/ml

Genotoxic effects:	clastogenicity			polyploidy		
	+	?	-	+	?	-
Without metabolic activation:	[*]	[]	[]	[]	[]	[*]
With metabolic activation:	[]	[]	[*]	[]	[]	[*]

- 1) The tests were performed by the Mitsubishi Chemical Safety Institute Ltd., 14 Sunayama, Hasaki-machi, Kashima-gun, Ibaraki, 31-02, Japan. Tel +81-479-46-2871 Fax +81-479-46-2874

Ministry of Health and Welfare: Japan, *Toxicity Testing Reports of Environmental Chemicals* 5, 359-386 (1997)

Glycidyl methacrylate

[CAS No. 106-91-2]

Molecular formula: C₇H₁₀O₃

Molecular weight: 142.17

ABSTRACT

2,3-Epoxypropyl Methacrylate was studied for oral toxicity in rats in an OECD combined repeat dose and reproductive/developmental toxicity screening test at doses of 0, 10, 30 and 100 mg/kg/day.

In the repeat dose study, salivation was observed in 5 male rats receiving 30 mg/kg and in all males (12 rats) receiving 100 mg/kg. Increased absolute and relative kidney weights were observed in both sexes receiving 100 mg/kg. Histopathologically, squamous hyperplasia of the forestomach was evident in males receiving 30 and 100 mg/kg, and edema of the forestomach submucosa was observed in males receiving 30 mg/kg. NOELs for repeat dose toxicity are considered to be 10 mg/kg/day for males and 30 mg/kg/day for females.

In terms of reproductive/developmental toxicity, the fertility index decreased significantly in the 100 mg/kg group. No effects were observed on the development of the next generation. NOELs for reproductive performance of both sexes, and pup development are considered to be 30 mg/kg/day and 100 mg/kg/day, respectively.

Genotoxicity of 2,3-epoxypropyl methacrylate was studied by chromosomal aberration test in cultured Chinese hamster lung (CHL/TU) cells. Structural chromosomal aberrations were induced under the following conditions: 24 h continuous treatment at 0.013 and 0.025 mg/ml (mid and high concentrations); 48 h continuous treatment at 0.025 mg/ml; short-term treatment with an exogenous metabolic activation system at 0.18 mg/ml (high concentration); short-term treatment without a metabolic activation system at 0.044 mg/ml (high concentration). Polyploidy was induced at 0.025 mg/ml with 48 h continuous treatment and at 0.044 mg/ml with short-term treatment without a metabolic activation system.

A significant and dose-dependent increase of micronucleated polychromatic erythrocytes (MNPCE) was observed in both male and female mice after 48 hours treatment. The proportion of polychromatic erythrocytes in the total erythrocytes was significantly lower at the highest dose in male and female mice.

SUMMARIZED DATA FROM THE STUDIES

1. Repeat Dose and Reproductive/Developmental Toxicity 1)

Purity: 99.93 %

Test species/strains: Rat/Crj: CD (SD)

Test method: OECD Combined Repeat Dose and Reproductive/Developmental Toxicity Screening Test

Route: Oral (gavage)

Doses: 0 (Vehicle), 10, 30, 100 mg/kg/day

Number of animals: Males, 12; females, 12/group

Vehicle: Corn oil

Administration period: Males, 45 days

Females, from 14 days before mating to day 3 of lactation

Terminal kill: Males, day 45

Females, day 4 of lactation

GLP: Yes

Test results:

<Repeat dose toxicity>

Salivation was observed in 5 male rats receiving 30 mg/kg and in all males (12 rats) receiving

100 mg/kg. There were no obvious influences of the test substance on the body weight gain or food consumption in either sex, or in the hematological and blood chemistry examinations of male rats. Increased absolute and relative kidney weights were observed in both sexes receiving 100 mg/kg. Pathological examination revealed no specific macroscopical findings attributable to the administration of the test substance. As histological findings, squamous hyperplasia of the forestomach was observed in males receiving 30 and 100 mg/kg, and edema of the forestomach submucosa was noted in males receiving 30 mg/kg. Many animals were infertile apparent in the 100 mg/kg group. However, morphological abnormalities were not apparent in the testes, ovaries, epididymis, seminal vesicles, prostate, uterus or pituitary gland. Moreover, counts of Stage VIII seminiferous tubules in the testes of the 100 mg/kg group did not reveal any effects attributable to the administration of the test substance.

NOELs for repeat dose toxicity are considered to be 10 mg/kg/day for males and 30 mg/kg/day for females.

<Reproductive and developmental toxicity>

The fertility index decreased significantly in the 100 mg/kg group, presumably due to the low sperm motility revealed by secondary investigations. There were no effects of the test substance on the estrous cycle, copulation index, gestation length, or parturition. Slight decreases in the numbers of corpora lutea, implants, pups born and live pups as well as the implantation and delivery indices were observed in the 100 mg/kg group. However, clear effects attributable to the administration of the test substance could not be concluded because of the few cases. There were no significant differences in the gestation index, live birth index or viability index on day 4. No abnormalities attributable to the administration of the test substance were noted in the body weights of live pups or on necropsy of pups in any treated group.

NOELs for reproductive performance of males and females, and pup development are considered to be 30 mg/kg/day and 100 mg/kg/day respectively.

2. Genetic Toxicity

2-1. Non-bacterial in vitro test (chromosomal aberration test)²⁾

Purity: 99.93 %

Type of cell used: Chinese hamster lung (CHL/IU) cells

Test method: Guidelines for Screening Mutagenicity Testing of Chemicals (Japan)

Solvent: Dimethylsulfoxide

Positive controls: -S9 mix, Mitomycin C

+S9 mix, Cyclophosphamide

Doses: - S9 mix (continuous treatment): 0, 0.0063, 0.013, 0.025 mg/ml

-S9 mix (short-term treatment): 0, 0.011, 0.022, 0.044 mg/ml

+S9 mix (short-term treatment): 0, 0.044, 0.088, 0.18 mg/ml

S-9: Rat liver, induced with phenobarbital and 5,6-benzoflavone

Plates/test: 2

GLP: Yes

Test results:

Cytogenetic effects were seen as follows.

Structural chromosomal aberrations (including gap) were induced under the following conditions: 24 h continuous treatment (0.013 and 0.025 mg/ml: mid and high concentrations, 3.5 and 89.5%); 48 h continuous treatment (0.025 mg/ml, 48.5 %); short-term treatment with an exogenous metabolic activation system (0.18 mg/ml: high concentration, 9.5%); short-term treatment without the metabolic activation system (0.044 mg/ml: high concentration, 28.5%). Polyploidy was induced under the following conditions: 24 h continuous treatment (0.013 mg/ml, 1.38%); 48 h continuous treatment (0.025 mg/ml, 9.40%); short-term treatment with an exogenous metabolic activation system (0.088 mg/ml: mid concentration, 0.88%); short-term treatment without the metabolic activation system (0.044 mg/ml, 0.88%). However, a trend test showed no dose-dependency for the induction of polyploidy with the 24 h continuous treatment and the short-

term treatment with the metabolic activation system.

Lowest concentration producing cytogenetic effects in vitro:

Without metabolic activation (continuous treatment)

: 0.013 mg/ml (clastogenicity)

: 0.025 mg/ml (clastogenicity and polyploidy)

Without metabolic activation (short-term treatment)

: 0.044 mg/ml (clastogenicity and polyploidy)

With metabolic activation (short-term treatment)

: 0.18 mg/ml (clastogenicity)

: 0.088 mg/ml (polyploidy)

Genotoxic effects:

	clastogenicity			polyploidy		
	+	?	-	+	?	-
Without metabolic activation:	[*]	[]	[]	[*]	[]	[]
With metabolic activation:	[*]	[]	[]	[]	[*]	[]

2-2. Non-bacterial in vivo test (Micronucleus test)2)

Purity: 99.93 %

Test species/strains: Mice/Crj: BDF1, male and female

Test methods: Guideline for Screening Mutagenicity Testing of Chemicals (Japan) and OECD Guideline No. 474

Procedure: Bone marrow/acridine orange staining

Solvent: Olive oil

Positive control: Cyclophosphamide 50 mg/kg

Doses: 0, 188, 375 and 750 mg/kg in males

0, 250, 500 and 1000 mg/kg in females

Mice/group: 5 male and f. male/group

GLP: Yes

Test results:

The frequency of micronucleated polychromatic erythrocytes was significantly increased in males and females with dose-dependency at 48h after oral gavage administration. Inhibition of bone marrow cell proliferation was observed at the high dose in both sexes under the test conditions.

Lowest dose producing toxicity: 400 mg/kg in males and females

Maximum tolerated dose: 750 mg/kg in males

1000 mg/kg in females

Genotoxic effect:

	+	?	-
Micronucleus test:	[*]	[]	[]

- 1) The tests were performed by the Biosafety Research Center, Food, Drugs and Pesticides (Anpyo Center), Japan, 582-2 Shioshinden Arahama, Fukuda-cho, Iwata-gun, Shizuoka, 437-12, Japan. Tel +81-538-58-1266 Fax +81-538-58-1393
- 2) The tests were performed by the Hatano Research Institute, Food and Drug Safety Center, 729-5 Ochiai, Hadano-shi, Kanagawa, 257, Japan. Tel +81-463-82-4751 Fax +81-463-82-9627

Ministry of Health and Welfare: Japan, *Toxicity Testing Reports of Environmental Chemicals 5*, 579-604 (1997)

Dimethylaminoethyl acrylate or 2-(Dimethylamino) ethyl acrylate

[CAS No. 2439-35-2]

Molecular formula: C₇H₁₃NO₂ Molecular weight: 143.21

ABSTRACT

2-(Dimethylamino) ethyl acrylate was studied for oral toxicity in SD(Crj:CD) rats in an OECD combined repeat dose and reproductive/developmental toxicity screening test at doses of 0, 4, 20 and 100 mg/kg/day.

With regard to repeat dose toxicity, two females died, and suppression of body weight gain and decrease in food consumption were observed in males in the 100 mg/kg group. Increase in reticulocyte, platelet and segmented neutrophil counts, and a decrease in albumin were also noted in this group. Histopathological examination revealed ulceration, inflammatory cell infiltration and hyperplasia of the mucosa in the forestomach, and hyperplasia of plasma cells in the pancreaticoduodenal lymph nodes in both sexes, and atrophy of the thymus in females of the same group. In the 20 mg/kg group, similar histopathological changes were observed in the forestomach in males. The NOELs for repeat dose toxicity are considered to be 4 mg/kg/day for males and 20 mg/kg/day for females. In terms of reproductive/developmental toxicity, the compound had no effects on any relevant parameters. The NOELs for reproductive/developmental toxicity are considered to be 100 mg/kg/day for parental animals and offspring.

2-(Dimethylamino) ethyl acrylate was mutagenic in *Salmonella typhimurium* TA98, with an exogenous metabolic activation system.

Genotoxicity of 2-(dimethylamino) ethyl acrylate was studied by chromosomal aberration test in cultured Chinese hamster lung (CHL/IU) cells. Structural chromosomal aberrations were induced with continuous treatment at 0.060 mg/ml (high concentration), and short-term treatment with and without an exogenous metabolic activation system at 0.050 and 0.010 mg/ml (both high concentrations), respectively. Polyploidy was induced with continuous treatment at 0.060 mg/ml, short-term treatment with the metabolic activation system at 0.025 and 0.050 mg/ml (low and high concentrations), and short-term treatment without metabolic activation system at 0.0050 and 0.010 mg/ml (low and high concentrations).

SUMMARIZED DATA FROM THE STUDIES

1. Repeat Dose and Reproductive/Developmental Toxicity1)

Purity: 99.9 %

Test species/strain: Rats/Crj: CD (SD)

Test method: OECD Combined Repeat Dose and Reproductive/Developmental Toxicity Screening Test

Route: Oral (gavage)

Dosage: 0 (Vehicle), 4, 20, 100 mg/kg/day

Number of animals: Males, 12; females, 12/group

Vehicle: Corn oil

Administration period: Males, 43 days

Females, from 14 days before mating to day 3 of lactation

Terminal kill: Males, day 44

Females, day 4 of lactation

GLP: Yes

Test results:

<Repeat dose toxicity>

In the 100 mg/kg group, two females died, and males showed a transient suppression of body weight gain and a decrease in food consumption. At necropsy, thickening of the wall of the forestomach and enlargement of the pancreatico-duodenal lymph nodes were observed in both sexes. Histopathological examination revealed ulceration, inflammatory cell infiltration and hyperplasia of the mucosa in the forestomach, and hyperplasia of plasma cells in the pancreatico-duodenal lymph nodes in both sexes. Atrophy of the thymus was also observed in females. Hematological and blood chemical examinations in males showed increased reticulocyte, platelet and segmented neutrophil counts, and a decrease in albumin. In the 20 mg/kg group, similar histopathological changes were observed in the forestomach in males.

The NOELs for repeat dose toxicity are considered to be 4 mg/kg/day for males and 20 mg/kg/day for females.

<Reproductive and developmental toxicity>

The compound had no effects on reproductive parameters such as the mating index, the fertility index, number of corpora lutea or implantations, the implantation index, the gestation index, the delivery index, gestation length, parturition or maternal behavior. On examination of neonates, there were no significant differences in number of offspring or live offspring, the sex ratio, the live birth index, the viability index or body weight. No abnormal findings ascribable to the compound were found for external features, clinical signs or necropsy of the offspring.

The NOELs for reproductive and developmental toxicity are considered to be 100 mg/kg/day for parental animals and offspring.

2. Genetic Toxicity

2-1. Bacterial test □ □

Purity: 99.9 wt%

Test species/strains: *Salmonella typhimurium* TA100, TA1535, TA98, TA1537, *Escherichia coli* WP2 uvrA

Test method: Guidelines for Screening Mutagenicity Testing of Chemicals (Japan) and OECD Guideline No. 471 and 472

Procedures: Pre-incubation method

Solvent: Water

Positive controls: -S9 mix, 2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide (TA100, TA98, WP2), Sodium azide (TA1535) and 9-Aminoacridine (TA1537)
+S9 mix, 2-Aminoanthracene (five strains)

Doses: -S9 mix;

0, 78.1, 156, 313, 625, 1250, 2500 µg/plate (TA98, TA1537)

0, 156 - 5000 µg/plate (TA100, TA1535, WP2)

+S9 mix;

0, 156 - 5000 µg/plate (TA100, TA1535, TA98, TA1537)

0, 313 - 5000 µg/plate (WP2)

S9: Rat liver, induced with phenobarbital and 5,6-benzoflavone

Plates/test: 3

Number of replicates: 2

GLP: Yes

Test results:

This chemical induced mutations in *S. typhimurium* TA98 with an S9 mix. Toxicity was observed at 1250 µg/plate (TA98, TA1537), 2500 µg/plate (TA1535), 5000 µg/plate (TA100, WP2) without S9 mix, and at 2500 µg/plate (TA1535), 5000 µg/plate (TA100, TA98, TA1537) with an S9 mix. Toxicity was not observed in WP2 with an S9 mix.

Genetic effects:

Salmonella typhimurium TA98

	+	?	-
Without metabolic activation	[]	[]	[*]
With metabolic activation	[*]	[]	[]
<i>Salmonella typhimurium</i> TA100, TA1535, TA1537			
	+	?	-
Without metabolic activation	[]	[]	[*]
With metabolic activation	[]	[]	[*]
<i>Escherichia coli</i> WP2 uvrA			
	+	?	-
Without metabolic activation	[]	[]	[*]
With metabolic activation	[]	[]	[*]

2-2. Non-bacterial in vitro test (chromosomal aberration test)2)

Purity: 99.9 wt%

Type of cell used: Chinese hamster lung (CHL/IU) cells

Test method: Guidelines for Screening Mutagenicity Testing of Chemicals (Japan) and OECD Guideline No. 473

Solvent: Distilled water

Positive controls: -S9 mix, Mitomycin C

+S9 mix, Cyclophosphamide

Doses: -S9 mix (continuous treatment): 0, 0.015, 0.030, 0.060 mg/ml

-S9 mix (short-term treatment): 0, 0.0050, 0.010 mg/ml

+S9 mix (short-term treatment): 0, 0.025, 0.050 mg/ml

S-9: Rat liver, induced with phenobarbital and 5,6-benzoflavone

Plates/test: 2

GLP: Yes

Test results:

Cytogenetic effect were seen as follows.

Structural chromosomal aberrations (including gap) were induced under the following conditions: 24 h continuous treatment (0.060 mg/ml: high concentrations, 23.5%); 48 h continuous treatment (0.060 mg/ml, 8.5%); short-term treatment with an exogenous metabolic activation system (0.050 mg/ml: high concentration, 12.5%); short-term treatment without the metabolic activation system (0.010 mg/ml: high concentration, 16.0%). Polyploidy was induced under the following conditions: 24 h continuous treatment (0.060 mg/ml, 10.75%); 48 h continuous treatment (0.060 mg/ml, 6.21%); short-term treatment with an exogenous metabolic activation system (0.025 and 0.050 mg/ml: low and high concentrations, 1.25 and 5.25%); short-term treatment without the metabolic activation system (0.0050 and 0.010 mg/ml: low and high concentrations, 1.25 and 10.88%).

Lowest concentration producing cytogenetic effects in vitro:

Without metabolic activation (continuous treatment): 0.060 mg/ml (clastogenicity)
: 0.060 mg/ml (polyploidy)

Without metabolic activation (short-term treatment): 0.010 mg/ml (clastogenicity)
: 0.0050 mg/ml (polyploidy)

With metabolic activation (short-term treatment): 0.050 mg/ml (clastogenicity)
: 0.025 mg/ml (polyploidy)

Genotoxic effects:	clastogenicity			polyploidy		
	+	?	-	+	?	-
Without metabolic activation:	[*]	[]	[]	[*]	[]	[]

With metabolic activation: [*] [] [] [*] [] []

- 1) The tests were performed by the Mitsubishi Chemical Safety Institute Ltd., 14 Sunayama, Hasaki-machi, Kashima-gun, Ibaraki, 314-02, Japan. Tel +81-47-46-2871 Fax +81-479-46-2874
- 2) The tests were performed by the Hatano Research Institute, Food and Drug Safety Center, 729-5 Ochiai, Hadano-shi, Kanagawa, 257, Japan. Tel +81-463-82-4751 Fax +81-463-82-9627

Ministry of Health and Welfare: Japan, *Toxicity Testing Reports of Environmental Chemicals* 6, 539-568 (1998)

2-(Dimethylamino)ethyl methacrylate
[CAS No. 2867-47-2]

Molecular formula: C₈H₁₅NO₂ Molecular weight: 157.24

ABSTRACT

A single dose oral toxicity test revealed an LD₅₀ value of above 2000 mg/kg for the compound in both sexes.

2-(Dimethylamino)ethyl methacrylate was studied for oral toxicity in rats in an OECD combined repeat dose and reproductive/developmental toxicity screening test at doses of 0, 40, 200 and 1000 mg/kg/day.

With regard to repeat dose toxicity, three females died in the 1000 mg/kg group. Soiled tail, twitching, chronic convulsion and suppression of body weight gain in both sexes, and a decrease in food consumption in females were also observed in the late period of administration in this group. Histopathological examination revealed degeneration of nerve fibers in the brain and spinal cord, and hyperplasia of the mucosa, edema and inflammatory cell infiltration in the forestomach in both sexes, and atrophy of the thymus in females in the 1000 mg/kg group. Increases in organ weights without histopathological changes were observed for the kidneys of both sexes, the livers of males, and the adrenals of females in this group. BUN was slightly increased in males in the same group. Slight anemic changes were observed in males of the 200 and 1000 mg/kg groups. The NOELs for repeat dose toxicity are considered to be 40 mg/kg/day for males and 200 mg/kg/day for females. In terms of reproductive/developmental toxicity, the compound exerted effects on maternal behavior, the body weight of neonates and the viability index in the 1000 mg/kg group. The NOELs for reproductive/developmental toxicity are considered to be 1000 mg/kg/day for parental males, and 200 mg/kg/day for parental females and offspring.

2-(Dimethylamino)ethyl methacrylate was mutagenic in *Salmonella typhimurium* TA1537 without an exogenous metabolic activation system.

2-(Dimethylamino)ethyl methacrylate induced structural chromosomal aberrations in CHL cells with and without an exogenous metabolic activation system.

SUMMARIZED DATA FROM THE STUDIES

1. Single Dose Toxicity1)

Purity: 99.8 %

Test species/strain: Rat/Crj: CD (SD)

Test method: OECD Test Guideline 401

Route: Oral (gavage)

Dosage: 0 (Vehicle), 500, 1000, 2000 mg/kg/day

Number of animals/group: Males, 5; females, 5

Vehicle: Corn oil

GLP: Yes

Test results:

No deaths occurred in any of the treated groups. At necropsy, raised patches in the forestomach were observed in males of the 2000 mg/kg group. Histopathologically, papillomatous hyperplasia in the forestomach was apparent.

2. Repeat Dose and Reproductive/Developmental Toxicity1)

Purity: 99.8 %

Test species/strain: Rat/Crj: CD (SD)

Test method: OECD Combined Repeat Dose and Reproductive/ Developmental Toxicity Screening

Test

Route: Oral (gavage)

Doses: 0 (vehicle), 40, 200, 1000 mg/kg/day

Number of animals/group: Males, 12; females, 12/group

Vehicle: Corn oil

Administration period: Males, 42 days

Female, from 14 days before mating to day 3 of lactation

Terminal kill: Males, 44 days

Females, day 4 of lactation

GLP: Yes

Test results:**<Repeat dose toxicity>**

In the 1000 mg/kg group, three females died. Soiled tail, twitching, chronic convulsion and suppression of body weight gain were observed in both sexes in the late period of administration. Food consumption was reduced in females during the lactation period. At necropsy, thickening of the wall of the forestomach was observed in both sexes. Histopathological examination revealed degeneration of nerve fibers in the brain and spinal cord, and hyperplasia of the mucosa, edema and inflammatory cell infiltration in the forestomach in both sexes. Atrophy of the thymus was also observed in females. Increases in organ weights without histopathological changes were observed for the kidneys of both sexes, the liver of males, and the adrenals in females. Slight increase of BUN in males was observed on blood chemical examination. Hematological examination in males showed slight anemic changes such as decreases in erythrocyte counts, hemoglobin concentration and hematocrit, and an increase in the reticulocyte ratio.

In the 200 mg/kg group, decreases in hemoglobin concentration and hematocrit were observed in males.

The NOELs for repeat dose toxicity are considered to be 40 mg/kg/day for males and 200 mg/kg/day for females.

<Reproductive and developmental toxicity>

The compound had no effects on reproductive parameters such as the mating index, the fertility index, numbers of corpora lutea or implantations, the implantation index, the delivery index, the gestation index, gestation length or parturition. Three dams of the 1000 mg/kg group, however, lost all their pups in the lactation period. On examination of neonates, the 1000 mg/kg dose was associated with a decrease in body weight and a low viability index. There were no significant differences in numbers of offspring or live offspring, the sex ratio or the live birth index. No abnormalities ascribable to the compound were found for external features, clinical signs or necropsy findings for the offspring.

The NOELs for reproductive and developmental toxicity are considered to be 1000 mg/kg/day for parental males, 200 mg/kg/day for parental females and offspring.

3. Genetic Toxicity**3-1. Bacterial test²⁾**

Purity: 99.8 %

Test species/strains: *Salmonella typhimurium* TA100, TA1535, TA98, TA1537, *Escherichia coli* WP2 uvrA

Test methods: Guidelines for Screening Mutagenicity Testing of Chemicals (Japan) and OECD Guidelines No. 471 and 472

Procedures: Pre-incubation method

Solvent: Distilled water

Positive controls: -S9 mix; 2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide (TA100, TA98 and WP2 uvrA), Sodium azide (TA1535), 9-Aminoacridine (TA1537)
+S9 mix, 2-aminoanthracene (all strains)

Doses: -S9 mix: 0, 156, 313, 625, 1250, 2500, 5000 µg/plate

+S9 mix: 0, 156, 313, 625, 1250, 2500, 5000 µg/plate

[Confirmative test]

+S9 mix: 0, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000 µg/plate

S9: Rat liver, induced with phenobarbital and 5,6-benzoflavone

Plates/test: 3

Number of replicates: 2

GLP: Yes

Test results:

This chemical induced mutations in *S. typhimurium* TA1537 without an S9 mix. Toxicity was observed at 5000 µg/plate (TA98, TA1537) without an S9 mix. In a confirmation test, toxicity was observed at more than 3500 µg/plate (TA98, TA1537) without an S9 mix.

Genotoxic effects:

Salmonella typhimurium TA1537

	+	?	-
Without metabolic activation	[*]	[]	[]
With metabolic activation	[]	[]	[*]

Salmonella typhimurium TA100, TA1535, TA98

	+	?	-
Without metabolic activation	[]	[]	[]
With metabolic activation	[]	[]	[]

Escherichia coli WP2 uvrA

	+	?	-
Without metabolic activation	[]	[]	[*]
With metabolic activation	[]	[]	[*]

3-2. Non-bacterial in vitro test (chromosomal aberration test)2)

Purity: 99.8 %

Type of cell used: Chinese hamster lung (CHL) cells

Test method: Guidelines for Screening Mutagenicity Testing of Chemicals (Japan) and OECD

Guideline No. 473

Solvent: Distilled water

Positive controls: -S9 mix, N-Methyl-N'-nitro-N-nitrosoguanidine

+S9 mix, Benzo[a]pyrene

Doses: -S9 mix (24 and 48-hr continuous treatment): 0, 20, 39, 78, 156, 313, 625 µg/mL

-S9 mix (6-hr short-term treatment): 0, 200, 400, 600, 800, 1400, 1600 µg/mL

+S9 mix (6-hr short-term treatment): 0, 200, 400, 600, 800, 1400, 1600 µg/mL

S-9: Rat liver, induced with phenobarbital and 5,6-benzoflavone

Plates/test: 2

GLP: Yes

Test results:

Structural chromosomal aberrations including gaps were induced at 625 µg/mL on 24- and 48-hr treatment (88.5 and 76.5 %, respectively) without an S9 mix, at 200, 400 and 600 µg/mL on 6-hr short-term treatment (6.5, 49.5 and 87.5 %) without an S9 mix, and at 800, 1400 and 1600 µg/mL on 6-hr short-term treatment (13.5, 99.5 and 100 %) with an S9 mix. Polyploidy was not induced in any treatment group.

Cytotoxicity was observed at more than 800 µg/mL on 6-hr short-term treatment without an S9 mix.

Genotoxic effects:	clastogenicity			polyploidy		
	+	?	-	+	?	-

Without metabolic activation: [*] [] [] [] [] [*]
With metabolic activation: [*] [] [] [] [] [*]

- 1) The tests were performed by the Mitsubishi Chemical Safety Institute Ltd., 14 Sunayama, Hasaki-machi, Kashima-gun, Ibaraki, 314-0255, Japan. Tel +81-479-46-2871 Fax +81-479-46-2874
- 2) The tests were performed by the Research Institute for Animal Science in Biochemistry and Toxicology, 3-7-11 Hashimoto-dai, Sagami-hara-shi, Kanagawa 229-1132, Japan. Tel +81-42-762-2775 Fax +81-42-762-7979

Ministry of Health and Welfare: Japan, *Toxicity Testing Reports of Environmental Chemicals* 6, 149-175 (1998)

2-(Diethylamino)ethyl methacrylate
[CAS No. 105-16-8]

Molecular formula: C₁₀H₁₉O₂N

Molecular weight: 185.27

ABSTRACT

2-(Diethylamino)ethyl methacrylate was studied for oral toxicity in rats in an OECD combined repeat dose and reproductive/developmental toxicity screening test at doses of 0, 50, 150 and 500 mg/kg/day.

With regard to repeat dose toxicity, high values for BUN and relative weights of kidneys were observed in males of the 150 mg/kg group and high values for BUN and absolute and relative weights of kidneys were observed in males of the 500 mg/kg group. Low values for hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration and high values for total iron-binding capacity and unsaturated iron-binding capacity were seen, as well as low a₂-globulin and high b₂-globulin fraction ratios which were thought to be reflections of the aforementioned changes. There were no effects of the test substance in females of the 500 mg/kg group.

The NOELs for repeat dose toxicity are considered to be 50 mg/kg/day for males, and 500 mg/kg/day for females.

With regard to reproductive/developmental toxicity, a tendency toward low numbers of pups and low delivery index were observed in the 500 mg/kg group. There were no effects of the test substance on development of pups.

The NOELs for reproductive performance are considered to be 500 mg/kg/day for males, 150 mg/kg/day for females, and 500 mg/kg/day for development of pups.

2-(Diethylamino)ethyl methacrylate was not mutagenic in *Salmonella typhimurium* TA100, TA1535, TA98, TA1537 and *Escherichia coli* WP2 uvrA, with or without an exogenous metabolic activation system.

Genotoxicity of 2-(diethylamino)ethyl methacrylate was studied by the chromosomal aberration test in cultured Chinese hamster lung (CHL/IU) cells.

2-(Diethylamino)ethyl methacrylate induced structural chromosomal aberrations at 0.30 and 0.60 mg/mL (mid and high concentrations) in the presence of an exogenous metabolic activation system. Polyploidy was induced at 0.15 and 0.30 mg/mL (mid and high concentrations) on continuous treatment for 24 h, at 0.30 mg/mL (high concentration) on continuous treatment for 48 h and at 0.30 mg/mL (high concentration) in the absence of an exogenous metabolic activation system.

SUMMARIZED DATA FROM THE STUDIES

1. Repeat Dose and Reproductive/Developmental Toxicity¹⁾

Purity: 99.8 %

Test species/strain: Rat/Crj: CD (SD)

Test method: OECD Combined Repeat Dose and Reproductive/ Developmental Toxicity Screening Test

Route: Oral (gavage)

Doses: 0 (vehicle), 50, 150 and 500 mg/kg/day

Number of animals/group: Males, 12; females, 12/group

Vehicle: Olive oil

Administration period: Males, 49 days

Females, from 14 days before mating to the day before autopsy (day 3 of lactation)

Terminal kill: Males, 50 days

Females, day 4 of lactation

GLP: Yes

Test results:

<Repeat dose toxicity>

In males of the 500 mg/kg group, low values for hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration and high values for unsaturated iron-binding capacity and total iron-binding capacity were seen, as well as low α_2 -globulin and high β -globulin fraction ratios, which were thought to be reflections of the aforementioned changes. In addition, high values for BUN and absolute and relative weights of kidneys were observed. There were no effects of the test substance on clinical signs, body weights, food consumption, urinalysis parameters, gross pathology or histopathology. In the 150 mg/kg group, high values for BUN and relative weights of kidneys were observed. There were no effects of the test substance on clinical signs, body weights, food consumption, urinalysis data, hematology, gross pathology or histopathology. In the 50 mg/kg group, there were no effects of the test substance with regard to any observation, measurement or examination. As for females, there were no effects of the test substance on clinical signs, body weights, food consumption or histopathology in any treatment groups.

The NOELs for repeat dose toxicity are considered to be 50 mg/kg/day for males, and 500 mg/kg/day for females.

<Reproductive/developmental toxicity>

With regard to male and female parents, there were no effects of the test substance on estrous cycle, copulation index, fertility index, length of gestation, delivery and lactation condition, the numbers of corpora lutea and implantation sites, implantation index or birth index. However, a tendency toward low numbers of pups born and a low delivery index were observed for the 500 mg/kg group.

With regard to pups, there were no effects of the test substance on sex ratio, the number of stillborn pups, live birth index, external anomalies, body weights, viability index on day 4 or gross pathology.

The NOELs for the reproductive/developmental toxicity are considered to be 500 mg/kg/day for males, 150 mg/kg/day for females, and 500 mg/kg/day for pups.

2-1. Bacterial test(2)

Purity: 99.8 %

Test species/strains: Salmonella typhimurium TA100, TA1535, TA98, TA1537, Escherichia coli WP2 uvrA

Test methods: Guidelines for Screening Mutagenicity Testing of Chemicals (Japan) and OECD Guidelines No. 471 and 472

Procedures: Pre-incubation method

Solvent: DMSO

Positive controls: -S9 mix, 2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide (TA100, TA98 and WP2 uvrA), Sodium azide (TA1535), 9-Aminoacridine hydrochloride (TA1537)
+S9 mix, 2-aminoanthracene (all strains)

Doses: -S9 mix: 0, 156, 313, 625, 1250, 2500, 5000 μ g/plate (TA100, TA1535, TA98 and TA1537); 0, 313 - 5000 μ g/plate (WP2 uvrA)
+S9 mix: 0, 156 - 5000 μ g/plate (five strains)

S9: Rat liver, induced with phenobarbital and 5,6-benzoflavone

Plates/test: 3

Number of replicates: 2

GLP: Yes

Test results:

This chemical did not induce gene mutations in *S. typhimurium* and *E. coli* strains. Toxicity was observed at 5000 μ g/plate (TA100, TA1535, TA98, TA1537) without an S9 mix and at 2500

µg /plate (five strains) with an S9 mix. Toxicity was not observed at 5000 µg /plate in WP2 uvrA without an S9 mix.

Genetic effects:

Salmonella typhimurium TA100, TA1535, TA98, TA1537

	+	?	-
Without metabolic activation	[]	[]	[*]
With metabolic activation	[]	[]	[*]

Escherichia coli WP2 uvrA

	+	?	-
Without metabolic activation	[]	[]	[*]
With metabolic activation	[]	[]	[*]

2-2. Non-bacterial in vitro test (chromosomal aberration test)2)

Purity: 99.8 %

Type of cell used: Chinese hamster lung (CHL) cells

Test method: Guidelines for Screening Mutagenicity Testing of Chemicals (Japan)and OECD Guideline No. 473

Solvent: DMSO

Positive controls: -S9 mix, Mitomycin C

+S9 mix, Cyclophosphamide

Doses: -S9 mix (continuous exposure): 0, 0.075, 0.15, 0.30 mg/mL

-S9 mix (short-term exposure): 0, 0.075, 0.15, 0.30 mg/mL

+S9 mix (short-term exposure): 0, 0.15, 0.30, 0.60 µg/mL

S-9: Rat liver, induced with phenobarbital and 5,6-benzoflavone

Plates/test: 2

GLP: Yes

Test results:

Cytogenetic effects were seen as follows.

Structural chromosomal aberrations (included gap) were induced at 0.30 and 0.60 mg/mL (mid and high concentrations, 6.0% and 24.5%, respectively) in the presence of an exogenous metabolic activation system. Polyploidy was induced under the following conditions: 24 h continuous treatment (0.15 and 0.30 mg/mL: mid and high concentration, 1.75 and 7.25%, respectively); 48 h continuous treatment (0.30 mg/mL, 5.13%); short-term treatment without an exogenous metabolic activation system (0.30 mg/mL, 7.13%).

Lowest concentration producing cytogenetic effects in vitro:

Without metabolic activation (continuous treatment): 0.15 mg/mL (polyploidy)

Without metabolic activation (short-term treatment): 0.30 mg/mL (polyploidy)

With metabolic activation (short-term treatment): 0.30 mg/mL (clastogenicity)

Genotoxic effects:	clastogenicity			polyploidy		
	+	?	-	+	?	-
Without metabolic activation:	[]	[]	[*]	[*]	[]	[]
With metabolic activation:	[*]	[]	[]	[]	[]	[*]

1) The tests were performed by In vivo Research Center Inc, 1284, Kamado, Gotemba-shi, Shizuoka, 412-0039, Japan. Tel +81-550-82-2000 Fax +81-550-82-2379

2) The tests were performed by the Hatano Research Institute, Food and Drug Safety Center, 729-5 Ochiai, Hadano-shi, Kanagawa, 257-0025, Japan. Tel +81-463-82-4751 Fax +81-463-82-9627

Ministry of Health and Welfare: Japan, *Toxicity Testing Reports of Environmental Chemicals* 6, 399-430 (1998)

2-Ethylhexyl methacrylate

[CAS No. 688-84-6]

Molecular formula: C₁₂H₂₂O₂

Molecular weight: 198.34

ABSTRACT

A single oral dose toxicity test revealed an LD₅₀ value of more than 2000 mg/kg for both sexes.

2-Ethylhexyl methacrylate was studied for oral toxicity in rats in an OECD combined repeat dose and reproductive/developmental toxicity screening test at doses of 0, 30, 100, 300 and 1000 mg/kg/day. One female died in the 1000 mg/kg group (12 animals of each sex).

In the males, the absolute kidney weights and the relative pituitary, liver and kidney weights were increased in the 300 and 1000 mg/kg groups. Suppression of body weight gain, decreased food consumption, RBC, hemoglobin, hematocrit, WBC and total protein, and increased GOT, GPT, A/G ratio, BUN and Cl, as well as focal necrosis in the liver and decreased extramedullary hematopoiesis in the spleen were seen in the 1000 mg/kg group. In the females, the relative kidney weights were increased in the 100 mg/kg or more groups. Suppression of body weight gain during the pre-mating, pregnancy and lactation periods and decrease in food consumption during the pre-mating period, atrophy of the thymus, increased absolute kidney, relative thyroid and liver weights, and decreased extramedullary hematopoiesis in the spleen and focal malacia in the medulla oblongata were seen in the 1000 mg/kg group. The NOELs for repeat dose toxicity are considered to be 100 mg/kg for males, and 30 mg/kg for females.

With regard to reproductive/developmental toxicity, three of the seven females lost their pups in the 1000 mg/kg group. Numbers of corpora lutea and implantation scars were decreased in the 1000 mg/kg group. The NOELs for reproductive performance are considered to be 1000 mg/kg for males, and 300 mg/kg for females. With regard to pups, decreased numbers of live pups born were seen in the 300 and 1000 mg/kg groups. Decreased birth, live birth and viability indices, and decreased body weights of both sexes on day 0 and day 4 after birth were seen in the 1000 mg/kg group. The NOEL for pup development is considered to be 100 mg/kg.

2-Ethylhexyl methacrylate was not mutagenic in *Salmonella typhimurium* TA100, TA1535, TA98, TA1537 and *Escherichia coli* WP2uvrA.

2-Ethylhexyl methacrylate did not induce structural chromosomal aberrations or polyploidy in CHL/TU cells, with or without an exogenous metabolic activation system.

SUMMARIZED DATA FROM THE STUDIES

1. Single Dose Oral Toxicity¹⁾

Purity: 99.4 %

Test species/strain: Rat/Crj: CD (SD)

Test method: OECD Test Guideline 401

Route: Oral (gavage)

Dosage: 0 (Vehicle), 500, 1000, 2000 mg/kg/day

Number of animals/group: Males, 5; females, 5

Vehicle: Corn oil

GLP: Yes

Test results:

No deaths occurred in any group.

Based on the above results, the LD₅₀ value of 2-ethylhexyl methacrylate was concluded to be more than 2000 mg/kg for both sexes.

2. Repeat Dose and Reproductive/Developmental Toxicity1)**Purity: 99.4 %****Test species/strain: Rat/Crj: CD (SD)****Test method: OECD Combined Repeat Dose and Reproductive/ Developmental Toxicity Screening Test****Route: Oral (gavage)****Doses: 0(vehicle), 30, 100, 300, 1000 mg/kg/day****Number of animals/group: Males, 12; females, 12/group****Vehicle: Corn oil****Administration period: Males, 49 days****Female, from 14 days before mating to day 3 of lactation****Terminal kill: Males, 50 days****Females, day 4 of lactation****GLP: Yes****Test results:****<Repeat Dose Toxicity>**

For the males, the absolute kidney weights and the relative pituitary, liver and kidney weights were increased in the 300 and 1000 mg/kg groups. Suppression of body weight gain and decreased food consumption, RBC, hemoglobin, hematocrit, VJC and total protein, and increased GOT, GPT, A/G ratio, BUN and Cl, as well as focal necrosis in the liver and decrease of extramedullary hematopoiesis in the spleen were seen in the 1000 mg/kg group.

For the females, the relative kidney weights were increased in the 100 mg/kg or more groups. Suppression of body weight gain during the pre-mating, pregnancy and lactation periods, decrease in food consumption during the pre-mating period, atrophy of the thymus, the increased absolute kidney, relative thyroid and liver weights, and decrease of extramedullary hematopoiesis in the spleen and focal malacia in the medulla oblongata were seen in the 1000 mg/kg group, one animal of which died.

The NOELs for repeat dose toxicity are considered to be 100 mg/kg for males, and 30 mg/kg for females.

<Reproductive/Developmental Toxicity>

With regard to reproductive/developmental toxicity, three of the seven females lost their pups in the 1000 mg/kg group. Nos. of corpora lutea and implantation scars were decreased in the 1000 mg/kg group. The NOELs for reproductive performance are considered to be 1000 mg/kg for males, and 300 mg/kg for females.

With regard to pups, decreased numbers of live pups born were seen in the 300 and 1000 mg/kg groups. Decreased birth, live birth and viability indices, and decreased body weights of both sexes on day 0 and day 4 after birth were seen in the 1000 mg/kg group. The NOEL for pup development is considered to be 100 mg/kg.

3-1. Bacterial test2)**Purity: 99.4 %****Test species/strains: Salmonella typhimurium TA100, TA1535, TA98, TA1537, Escherichia coli WP2 uvrA****Test methods: Guidelines for Screening Mutagenicity Testing of Chemicals (Japan) and OECD Guidelines No. 471 and 472****Procedures: Pre-incubation method****Solvent: DMSO****Positive controls: -S9 mix; 2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide (TA100, TA98 and WP2 uvrA), Sodium azide (TA1535), 9-Aminoacridine (TA1537)
+S9 mix, 2-aminoanthracene (all strains)****Doses: -S9 mix: 0, 39.1, 19.5, 9.77, 4.88, 2.44, 1.22, 0.610 µg/plate (TA100, TA1535, TA1537);**

313, 625, 1250, 2500, 5000 µg/plate (WP2 uvrA, TA98)
 +S9 mix: 0, 625, 313, 156, 78.1, 39.1, 19.5, 9.77 µg/plate (TA100, TA1535, TA1537);
 39.1, 78.1, 156, 313, 625, 1250, 2500 µg/plate (WP2 uvrA, TA98)

S9: Rat liver, induced with phenobarbital and 5,6-benzoflavone

Plates/test: 3

Number of replicates: 2

GLP: Yes

Test results:

This chemical did not induce gene mutations in the *S. typhimurium* and *E. coli* strains. Toxicity was observed at concentrations of 9.77 µg/plate (TA100, TA1535 and TA1537) without metabolic activation, and 156 µg/plate (TA100 and TA1535) and 625 µg/plate (WP2 uvrA, TA98) and 313 µg/plate (TA1537) with metabolic activation.

Genotoxic effects:

	<i>Salmonella typhimurium</i> TA100, TA1535, TA98 TA1537		
	+	?	-
Without metabolic activation	[]	[]	[*]
With metabolic activation	[]	[]	[*]
	<i>Escherichia coli</i> WP2 uvrA		
	+	?	-
Without metabolic activation	[]	[]	[*]
With metabolic activation	[]	[]	[*]

3-2. Non-bacterial in vitro test (chromosomal aberration test)2)

Purity: 99.4 %

Type of cell used: Chinese hamster lung (CHL) cells

Test method: Guidelines for Screening Mutagenicity Testing of Chemicals (Japan) and OECD Guideline No. 473

Solvent: Acetone

Positive controls: -S9 mix, N-Methyl-N'-nitro-N-nitrosoguanidine
 +S9 mix, Benzo[a]pyrene

Doses: -S9 mix (24 hr continuous treatment): 0, 10, 20, 40, 80 µg/mL

-S9 mix (48 hr continuous treatment): 0, 10, 20, 40, 80 µg/mL

-S9 mix (6 hr short-term treatment): 0, 10, 20, 40, 80 µg/mL

+S9 mix (6 hr short-term treatment): 0, 0, 625, 1250, 2500, 5000 µg/mL

S-9: Rat liver, induced with phenobarbital and 5,6-benzoflavone

Plates/test: 2

GLP: Yes

Test results:

This chemical did not induce structural chromosomal aberrations or polyploidy under the conditions of this experiment.

Genotoxic effects:	clastogenicity			polyploidy		
	+	?	-	+	?	-
Without metabolic activation:	[]	[]	[*]	[]	[]	[*]
With metabolic activation:	[]	[]	[*]	[]	[]	[*]

1) The test was performed by Nihon Bioresearch Inc. Hashima Laboratory, 6-104 Majima, Fukujicho, Hashima, Gifu, 501-6251, Japan Tel +81-58-392-6222 Fax +81-58-391-3171

2) The tests were performed by the Mitsubishi Chemical Safety Institute Ltd., 14 Sunayama, Hasaki-machi, Kashima-gun, Ibaraki, 314-0255, Japan. Tel +81-479-46-2871 Fax +81-479-46-

Ministry of Health and Welfare: Japan, *Toxicity Testing Reports of Environmental Chemicals 4*, 561-586 (1996)

2-Hydroxypropyl methacrylate

[CAS No. 923-26-2]

Molecular formula: C₇H₁₂O₃ Molecular weight: 144.17

ABSTRACT

2-Hydroxypropyl methacrylate was studied for its oral toxicity in rats in a single dose toxicity test at doses of 0, 500, 1000 and 2000 mg/kg. The single dose oral toxicity test revealed an LD50 value of more than 2000 mg/kg for both sexes.

2-Hydroxypropyl methacrylate was studied for its oral toxicity in rats in an OECD combined repeat dose and reproductive/developmental toxicity screening test at doses of 0, 30, 100, 300 and 1000 mg/kg/day. With regard to repeat dose toxicity, for the males, salivation, decreases in locomotor activity, ptosis, hematocrit, RBC and hemoglobin and an increase in the relative liver weight were seen at 1000 mg/kg, and 2 of the 12 animals died. For the females, salivation, decreases in locomotor activity and ptosis were seen at 1000 mg/kg, and 1 of the 12 animals died. The NOEL for repeat dose toxicity is considered to be 300 mg/kg for both sexes. With regard to reproductive/developmental toxicity, no effects of the test substance on copulation, fertility, delivery as lactation were noted. The NOELs for reproductive performance of males and females, and for pup development are considered to be 1000 mg/kg/day for both sexes.

2-Hydroxypropyl methacrylate was not mutagenic to *Salmonella typhimurium* TA100, TA98, TA1535, TA1537 and *Escherichia coli* WP2 uvrA, with or without an exogenous metabolic activation system.

2-Hydroxypropyl methacrylate induced structural chromosomal aberrations in CHL/IU cells with and without an exogenous metabolic activation system. Polyploidy was induced without an exogenous metabolic activation system.

SUMMARIZED DATA FROM THE STUDIES

1. Single Dose Oral Toxicity 1)

Purity: 98%

Test species/strain: Rat/Crj: CD (SD)

Test method: OECD Guidelines 401

Route: Oral (gavage)

Doses: 0 (vehicle), 500, 1000, 2000 mg/kg

Number of animals/group: Males, 5; females, 5

Vehicle: Water for injection

GLP: Yes

Test results:

No deaths occurred in any group. Males of the 2000 mg/kg group demonstrated salivation immediately after administration.

Based on the above results, the LD50 value of 2-hydroxypropyl methacrylate was concluded to be 2000 mg/kg or more for both sexes.

2. Repeat Dose and Reproductive/Developmental Toxicity 1)

Purity: 98%

Test species/strain: Rat/Crj: CD (SD)

Test method: OECD Combined Repeat Dose and Reproductive/Developmental Toxicity Screening Test

Route: Oral (gavage)

Doses: 0 (vehicle), 30, 100, 300, 1000 mg/kg/day

Number of animals/group: Males, 12; females, 12

Vehicle: Water for injection

Administration period: Males, 49 days

Females, from 14 days before mating to day 3 of lactation

Termination: Males, day 50

Females, day 4 of lactation

GLP: Yes

Test results:

<Repeat Dose Toxicity>

For the males, salivation, decrease in locomotor activity and ptosis were found in the 1000 mg/kg group, and 2 animals of the group died. Decrease in hematocrit, tendencies for decrease in RBC and hemoglobin, and increase in the relative liver weights were also found in the 1000 mg/kg group.

For the females, salivation, decrease in locomotor activity and ptosis were found in the 1000 mg/kg group, and 1 animal died.

The NOEL for the repeated dose toxicity is considered to be 300 mg/kg/day for both sexes.

<Reproductive/Developmental Toxicity>

There were no effects of the test substance on the estrus frequency, copulation index, number of days to conception, fertility index, length of gestation, number of corpora lutea or gestation index.

There were no effects of the test substance on the number of live pups born, birth index, number of dead pups, number of pups born, delivery index, live birth index, sex ratio, viability index, external anomalies, body weight or necropsy findings.

The NOELs for the reproductive/developmental toxicity are considered to be 1000 mg/kg/day for reproduction in both sexes as well as for development of pups.

3. Genetic Toxicity

3-1. Bacterial test 2)

Purity: 98%

Test species/strains: *S. typhimurium* TA100, TA1535, TA98, TA1537, *Escherichia coli* WP2 uvrA

Test methods: Guidelines for Screening Mutagenicity Testing of Chemicals (Japan) and OECD (471 and 472)

Procedures: Plate incorporation method

Solvent: Acetone

Positive controls: -S9 mix, 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide (TA100, WP2, TA98),

Sodium azide (TA1535) and 9-Aminoacridine (TA1537),

+S9 mix, 2-Aminoanthracene (five strains)

Dosage: 0, 313, 625, 1250, 2500 and 5000 µg/plate in five strains, -S9 mix and +S9 mix

S9: Rat liver, induced with phenobarbital and 5,6-benzoflavone

Plates/test: 3

Number of replicates: 2

GLP: Yes

Test results:

This chemical did not induce gene mutations in the *S. typhimurium* and *E. coli* strains. No toxicity was observed in the five strains with either the -S9 mix or the +S9 mix.

Genetic effects:

Salmonella typhimurium TA100, TA1535, TA98, TA1537

	+	?	-
Without metabolic activation	[]	[]	[*]
With metabolic activation	[]	[]	[*]

Escherichia coli WP2 uvrA

	+	?	-
Without metabolic activation	[]	[]	[*]
With metabolic activation	[]	[]	[*]

3-2. Non-bacterial in vitro test (chromosomal aberration test) 2)

Purity: 98%

Type of cell used: Chinese hamster lung (CHL/IU) cells

Test method: Guidelines for Screening Mutagenicity Testing of Chemicals (Japan)

Solvent: Acetone

Positive controls: -S9 mix, Mitomycin C

+S9 mix, Cyclophosphamide

Doses: -S9 mix (continuous treatment): 0, 0.18, 0.35, 0.70 mg/ml

-S9 mix (short-term treatment): 0, 0.35, 0.70, 1.4 mg/ml

+S9 mix (short-term treatment): 0, 0.35, 0.70, 1.4 mg/ml

S-9: Rat liver, induced with phenobarbital and 5,6-benzoflavone

Plates/test: 2

GLP: Yes

Test results:

2-Hydroxypropyl methacrylate induced structural chromosomal aberrations in CHL/IU cells with and without an exogenous metabolic activation system. Polyploidy was induced without an exogenous metabolic activation system.

Lowest concentration producing cytogenetic effects in vitro:

Without metabolic activation (continuous treatment): 0.35 mg/ml

(clastogenicity)

0.18 mg/ml (polyploidy)

Without metabolic activation (short-term treatment): 1.4 mg/ml (clastogenicity)

With metabolic activation (short-term treatment): 0.35 mg/ml (clastogenicity and polyploidy)

Genotoxic effects:	clastogenicity			polyploidy		
	+	?	-	+	?	-
Without metabolic activation:	[*]	[]	[]	[*]	[]	[]
With metabolic activation:	[*]	[]	[]	[*]	[]	[]

1) The test was performed by Nihon Bioresearch Inc. Hashima Laboratory, 6-104 Majima, Fukuju-cho, Hashima, Gifu, 501-62 Japan Tel +81-58-392-6222 Fax +81-58-391-3171

2) The tests were performed by the Hatano Research Institute, Food and Drug Safety Center, 729-5 Ochiai, Hadano-shi, Kanagawa, 257, Japan. Tel +81-463-82-4751 Fax +81-463-82-9627

Ministry of Health and Welfare: Japan, *Toxicity Testing Reports of Environmental Chemicals 5*, 525-552 (1997)

2-Hydroxyethyl methacrylate

[CAS No. 868-77-9]

Molecular formula: C₆H₁₀O₃

Molecular weight: 130.16

ABSTRACT

2-Hydroxyethyl methacrylate was studied for oral toxicity in rats in an OECD combined repeat dose and reproductive/developmental toxicity screening test at doses of 0, 30, 100, 300 and 1000 mg/kg/day. One male and 6 females of the 1000 mg/kg group (12 animals of each sex) died during the treatment period.

In the males, BUN was elevated or tended to be high at 30 mg/kg or more, and the relative kidney weights were increased at 100 mg/kg or more. Salivation, suppression of body weight gain, decrease in food consumption, increased K, Cl and inorganic phosphorus, decreased triglyceride, increased relative liver weights, dilatation of renal tubules and collection tubules in the kidney were seen at 1000 mg/kg. In the females, the relative kidney weights were elevated or tended to be high at 100 mg/kg or more. Salivation, decrease in locomotor activity, adoption of a prone position, lacrimation, soiled fur, hypothermia, bradypnea, suppression in body weight gain, decrease in food consumption, increases of the absolute and relative kidney weights, neutrophil cellular infiltration in the papilla and medulla and massive malacia in the medulla oblongata were seen at 1000 mg/kg. The NOELs for repeat dose toxicity are considered to be less than 30 mg/kg for males, and 30 mg/kg for females.

With regard to reproductive/developmental toxicity, no effects of the test substance on copulation, fertility, delivery or lactation were noted.

The NOELs for reproductive performance of males and females, and for pup development are considered to be 1000 mg/kg/day both.

2-Hydroxyethyl methacrylate was not mutagenic in *Salmonella typhimurium* TA100, TA1535, TA98, TA1537 and *Escherichia coli* WP2 uvrA, with or without an exogenous metabolic activation system.

Genotoxicity of 2-hydroxyethyl methacrylate was studied by the chromosomal aberration test in cultured Chinese hamster lung (CHL/IU) cells. Structural chromosomal aberrations were induced at 0.65 and 1.3 mg/ml (mid and high concentrations) with 24 h continuous treatment, at 0.16 - 0.65 mg/ml (all concentrations) with 48 h continuous treatment and at 1.3 mg/ml (high concentration) with short-term treatment and an exogenous metabolic activation system. Polyploidy was induced at 0.65 mg/ml with 48 h continuous treatment and at 0.33 and 1.3 mg/ml (low and high concentrations) with short-term treatment without the metabolic activation system.

SUMMARIZED DATA FROM THE STUDIES

1. Repeat Dose and Reproductive/Developmental Toxicity¹⁾

Purity: 97.6 %

Test species/strain: Rat/Crj: CD (SD)

Test method: OECD Combined Repeat Dose and Reproductive/Developmental Toxicity Screening Test

Route: Oral (gavage)

Dosage: 0 (vehicle), 30, 100, 300, 1000 mg/kg/day

Number of animals/group: Males, 12; females, 12

Vehicle: Distilled water

Administration period: Males, 49 days

Females, from 14 days before mating to day 3 of lactation

Terminal kill: Males, day 50

Females, day 4 of lactation

GLP: Yes

Test results:**<Repeat Dose Toxicity>**

For the males, BUN was elevated or tended to be high in the 30 mg/kg or more groups, the relative kidney weights were increased in the 100 mg/kg or more groups. Salivation, suppression of body weight gain, decreased food consumption, increased K, Cl and inorganic phosphorus, decreased triglyceride, increased relative liver weights, and dilatation of renal tubules and collection tubules in the kidney were found in the 1000 mg/kg group, 1 animal of which died.

For the females, the relative kidney weights were elevated or tended to be high in the 100 mg/kg or more groups. Salivation, decrease in locomotor activity, adoption of a prone position, lacrimation, soiled fur, hypothermia, bradypnea, suppression of body weight gain, decreased food consumption, increased absolute and relative kidney weights, neutrophil cellular infiltration in the papilla and medulla and massive malacia in the medulla oblongata were found in the 1000 mg/kg group, 6 animals of which died.

The NOELs for repeat dose toxicity are considered to be less than 30 mg/kg for males, and 30 mg/kg for females.

<Reproductive/Developmental Toxicity>

There were no effects of the test substance on the estrus frequency, copulation index, number of conceiving days, fertility index, length of gestation, number of corpora lutea or gestation index.

There were no effects of the test substance on the number of live pups born, birth index, number of dead pups, number of pups born, delivery index, live birth index, sex ratio, viability index, external anomalies, body weight or necropsy findings.

The NOELs for the reproductive/developmental toxicity are considered to be 1000 mg/kg/day for reproduction in both sexes as well as for development of pups.

2. Genetic Toxicity**2-1. Bacterial test²⁾**

Purity: 97.6 wt%

Test species/strains: *Salmonella typhimurium*, TA100, TA1535, TA98, TA1537, *Escherichia coli* WP2 uvrA

Test method: Guidelines for Screening Mutagenicity Testing of Chemicals (Japan) and OECD Guideline No. 471 and 472

Procedures: Pre-incubation method

Solvent: Water

Positive controls: -S9 mix, 2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide (TA100, TA98, WP2), Sodium azide (TA1535) and 9-Aminoacridine (TA1537)
+S9 mix, 2-Aminoanthracene (five strains)

Doses: -S9 mix; 0, 313, 625, 1250, 2500, 5000 µg/plate (five strains)

+S9 mix; 0, 313 - 5000 µg/plate (five strains)

S9: Rat liver, induced with phenobarbital and 5,6-benzoflavone

Plates/test: 3

Number of replicates: 2

GLP: Yes

Test results:

This chemical did not induce mutations in the *S. typhimurium* and *E. coli* strains. Toxicity was not observed at 5000 µg/plate in five strains with or without an S9 mix.

Genetic effects:

Salmonella typhimurium TA100, TA1535, TA98, TA1537

	+	?	-
Without metabolic activation	[]	[]	[*]
With metabolic activation	[]	[]	[*]

Escherichia coli WP2 uvrA

	+	?	-
Without metabolic activation	[]	[]	[*]
With metabolic activation	[]	[]	[*]

2-2. Non-bacterial in vitro test (chromosomal aberration test)²⁾

Purity: 97.6 %

Type of cell used: Chinese hamster lung (CHL/TU) cells

Test method: Guidelines for Screening Mutagenicity Testing of Chemicals (Japan) and OECD Guideline No. 473

Solvent: Distilled water

Positive controls: -S9 mix, Mitomycin C
+S9 mix, Cyclophosphamide

Doses: -S9 mix (continuous treatment): 0, 0.16, 0.33, 0.65, 1.3 mg/ml

-S9 mix (short-term treatment): 0, 0.33, 0.65, 1.3 mg/ml

+S9 mix (short-term treatment): 0, 0.33, 0.65, 1.3 mg/ml

S-9: Rat liver, induced with phenobarbital and 5,6-benzoflavone

Plates/test: 2

GLP: Yes

Test results:

Cytogenetic effects were seen as follows.

Structural chromosomal aberrations (including gap) were induced under the following conditions: 24 h continuous treatment (0.65 and 1.3 mg/ml: mid and high concentrations, 10.0 and 70.6 %, respectively); 48 h continuous treatment (0.16 - 0.65 mg/ml: all concentrations, 6.0 - 84.0 %); short-term treatment with an exogenous metabolic activation system (1.3 mg/ml: high concentration, 13.0 %). Polyploidy was induced under the following conditions: the 48 h continuous treatment (0.65 mg/ml, 3.25%); short-term treatment with an exogenous metabolic activation system (0.65 mg/ml: mid concentration, 1.25%); short-term treatment without the metabolic activation system (0.33 and 1.3 mg/ml: low and high concentrations, 0.38 and 6.13 %, respectively). However, a trend test showed no dose-dependency for the polyploidy with short-term treatment and the metabolic activation system.

Lowest concentration producing cytogenetic effects in vitro:

Without metabolic activation (continuous treatment): 0.16 mg/ml (clastogenicity)
: 0.65 mg/ml (polyploidy)

Without metabolic activation (short-term treatment): 0.33 mg/ml (polyploidy)

With metabolic activation (short-term treatment): 1.3 mg/ml (clastogenicity)
: 0.65 mg/ml (polyploidy)

Genotoxic effects:

	clastogenicity			polyploidy		
	+	?	-	+	?	-
Without metabolic activation:	[*]	[]	[]	[*]	[]	[]
With metabolic activation:	[*]	[]	[]	[]	[*]	[]

1) The test was performed by Nihon Bioresearch Inc. Hashima Laboratory, 6-104 Majima, Fukujicho, Hashima, Gifu, 501-62 Japan Tel +81-58-392-6222 Fax +81-58-391-3171

2) The tests were performed by the Hatano Research Institute, Food and Drug Safety Center, 729-5 Ochiai, Hadano-shi, Kanagawa, 257, Japan. Tel +81-463-82-4751 Fax +81-463-82-9627