Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

International Chemical Identification:

2-ethyl-2-[[(1-oxoallyl)oxy]methyl]-1,3-propanediyl diacrylate; 2,2-bis(acryloyloxymethyl)butyl acrylate;

trimethylolpropane triacrylate

EC Number: 239-701-3

CAS Number: 15625-89-5

Index Number: 607-111-00-9

Contact details for dossier submitter:

Version number: v1 **Date: February 2019**

Note on confidential information

Please be aware that this report is intended to be made publicly available. Therefore it should not contain any confidential information. Such information should be provided in a separate confidential Annex to this report, clearly marked as such.

CONTENTS

SUPPORT ON HOW TO COMPILE ANNEX I TO THE CLH REPORT

Annex I to the CLH report may be compiled from DARs, CARs and/or other sources. Non-confidential DAR/CAR can be annexed as such provided that it has sufficient level of details on the studies. The DS is encouraged to remove any irrelevant parts of the DAR/CAR. The DS must ensure that Annex I can be published during PC, i.e. it does not contain any confidential information.

For support, below is an example on how each study could be presented individually under its own subchapter including the study reference, detailed study summary and results. The format of the detailed study summary of an individual study is flexible as long as the summary is clearly reported and under a correct hazard class. Detailed support can be found below under each subchapter. If DAR/CAR is annexed to the CLH report as Annex I, it must be indicated clearly in the evaluation part of the report where in Annex I the relevant study can be found. If read-across to structurally or mechanistically similar substance is used please provide a justification for using data from this substance and, if known, present the calculations to convert dose/concentration levels from the test substance to the substance for which CLH is proposed. Please provide also a justification for providing non-testing data by any other approaches such as quantitative structure-activity relationships (QSARs) or grouping methods. Support on grouping of substances and read-across can be found in the following links:

http://echa.europa.eu/documents/10162/13632/information_requirements_r6_en.pdf

http://echa.europa.eu/documents/10162/13655/pg_report_qsars_en.pdf

http://echa.europa.eu/documents/10162/13655/pg_report_readacross_en.pdf

http://www.qsartoolbox.org/

http://www.oecd.org/chemicalsafety/risk-assessment/groupingofchemicalschemicalcategoriesandread-across.htm

http://echa.europa.eu/en/view-article/-/journal_content/title/assessing-read-across-how-echa-does-it

1 HEALTH HAZARDS

1.1 Germ cell mutagenicity

1.1.1 In vitro data

1.1.1.1 [Anonymous (1989)]

Study reference:

Anonymous (1989). Trimethylolpropantriacrylat (ZST Test Substance No.: 88/998) in the AMES TEST.

Detailed study summary and results:

Test type

Equivalent or similar to OECD Guideline 471 (Bacterial Reverse Mutation Assay)

- 3 plates per dose
- 0-20-100-500-2500-5000 µg/plate for experiment 1 (TA100, TA98), experiment 2 (TA1535, TA1537)
- 0-500-1000-2000-3000-4000 µg/plate for experiment 3 (TA1535)
- Negative control (solvent control, sterility control)
- Positive control: 2-aminoanthracene (TA100, 98, 1537, 1535 with S9 mix); N-methyl-N'-notro-N-nitroso-guanidine (TA100, 1535 without S9 mix); 9-aminoacridine chloride monohydrate (TA1537 without S9 mix); 4-nitro-o-phenylenediamine (TA98 without S9 mix)

Test substance

- TMPTA
- Test substance No.: 88/998
- Purity > 70%

Administration/exposure

- TA1535, TA100, TA1537, TA98
- Standard plate test with and without metabolic activation (S9 mix)
- Type and composition of metabolic activation system:
  - Male Sprague-Dawley rats received single IP injection of 500 mg Arochlor 1254 (20% solution in peanut oil – w/v) per kg body weight 5 days before sacrifice.
- Concentrations used: 20 µg – 5000 µg/plate
- Vehicle: DMSO (complete solubility)

Results and discussion

- A weakly bacteriotoxic effect was observed only using TA 98 with S-9 mix at doses >= 2500 µg/plate.
- Negative for TA 1537, TA98 and TA100 with metabolic activation.

- Positive (weakly positive reactions) for TA 1535 with metabolic activation but without dose-dependency over a dose range of 500-5000 µg/plate.

AMES TEST WITH : 88/998 DATE : 30.01.89
METHOD : STANDARD PLATE TEST STRAIN: TA 1535

DOSE MCG/PL	REVERTANTS / PLATE -S9	M	SD	+S9*	M	SD	TITER DIL. EXP-6	QUOTIENT -S9	+S9*
NEGATIVE CONTROL DMSO	16 19 20	18	2	23 21 18	21	3	28 35 41	1.0	1.0
20	17 17 20	18	2	22 22 24	23	1		1.0	1.1
100	23 20 20	21	2	24 29 27	27	3		1.1	1.3
500	21 21 15	19	3	36 30 35	34	3		1.0	1.6
2500	20 18 19	19	1	51 57 51	53	3	41 36 33	1.0	2.6
5000	18 22 19	20	2	47 42 46	45	3	25 34 26	1.1	2.2
POSITIVE CONTROL 2-AA 10				125 158 123	135	20			6.5
POSITIVE CONTROL MNNG	2080 2090 1920	2030	95					110.7	

- Negative for TA 1535, TA 1537, TA 98 and TA 100 without metabolic activation.
- Negative and positive control data: as expected

1.1.1.2 [Cameron et al., 1991]

Study reference:

Cameron TP, Rogers-Back AM, Lawlor TE, Harbell JW, Seifried HE, Dunkel VC. Genotoxicity of multifunctional acrylates in the Salmonella/mammalian-microsome assay and mouse lymphoma TK+/-assay. Environ Mol Mutagen. 1991;17(4):264-71.

Detailed study summary and results:

Ames test: Equivalent or similar to OECD Guideline 471

Mouse lymphoma TK+/- assay: Equivalent or similar to OECD Guideline 476

Test type

- Triplicate for Ames test and duplicate for MLA
- Solvent and positive and control groups included

Test substance

- TMPTA
- Purity = 79% (Morton Thiokol Alfa Products)

Administration/exposure

- Ames test
    - *S. typhimurium* TA 1535, TA 1537, TA 98 and TA 100 (met. act.: with and without)
    - Plate incorporation method
    - 100- 10000 µg/plate
- MLA:
    - Cells exposed ($12x10^6$ cells total) exposed for 4 hours. Incubation for 10-12 days and then counted with an automated colony counter. Mutant frequencies were expressed as mutants per 10^6 surviving cells.
    - 0.0005 – 100 µl/ml
- Type and composition of metabolic activation system:
    - Prepared from male Sprague-Dawley rats and Syrian golden hamsters (only for the Ames test) that had been injected with Arochlor 1254 at 500 mg/kg
- Vehicle: DMSO

Results and discussion

- Ames test
    - Doses selected on the basis of cytotoxicity observed in a dose range-finding assay using TA 100. If no toxicity observed, a maximum dose of 10 mg/plate was chosen.
    - Not cytotoxic
    - Negative for TA 1535, TA 1537, TA 98 and TA 100 without metabolic activation and with metabolic activation (rat S9)
    - Weak positive for TA 1535 with hamster S9
- MLA

- The doses selected for testing were within the range yelding approximately 0-90% cytotoxicity based on a toxicity study
- Cytotoxicity: yes (see table below: relative total growth)
- Negative for mouse lymphoma L5178Y cells with metabolic activation
- Positive for mouse lymphoma L5178Y cells without metabolic activation

- Vehicle and positive controls valid

TABLE II. Mutagenicity of Acrylate Compounds in the *Salmonella*/Mammalian-Microsome Assay

Dose	Average revertants/plate[a] ± standard deviation											
	TA 98			TA 100			TA 1535			TA 1537		
(μg/plate)	(−)S9	R(S9)	H(S9)	(−)S9	R(S9)	H(S9)	(−)S9	R(S9)	H(S9)	(−)S9	R(S9)	H(S9)
Trimethylolpropane triacrylate												
Solvent control	20 ± 4	25 ± 6	23 ± 1	96 ± 9	98 ± 10	77 ± 3	19 ± 6	10 ± 6	14 ± 2	6 ± 2	4 ± 2	8 ± 2
100	13 ± 3	29 ± 5	24 ± 4	104 ± 18	95 ± 5	85 ± 4	23 ± 2	9 ± 4	16 ± 7	3 ± 1	5 ± 1	6 ± 1
333	9 ± 3	23 ± 6	18 ± 3	90 ± 8	95 ± 8	82 ± 12	22 ± 1	15 ± 6	28 ± 6	3 ± 1	5 ± 2	6 ± 1
1,000	11 ± 2	21 ± 4	19 ± 5	104 ± 6	82 ± 4	78 ± 13	24 ± 2	16 ± 4	33 ± 5	4 ± 2	6 ± 3	4 ± 4
3,333	14 ± 3	15 ± 1	13 ± 2	67 ± 9	64 ± 15	73 ± 8	21 ± 1	16 ± 3	34 ± 2	2 ± 1	5 ± 2	3 ± 1
6,667								8 ± 3	11 ± 4			
10,000	7 ± 1	2 ± 1	2 ± 1	31 ± 8	3 ± 2	14 ± 10	11 ± 3			2 ± 1	0 ± 1	2 ± 1
Positive control	717 ± 39	431 ± 44	2,960 ± 143	1,342 ± 132	684 ± 21	3,036 ± 118	1,103 ± 37	253 ± 27	225 ± 13	302 ± 31	244 ± 20	187 ± 38

TABLE III. Mean TA1535 Revertants per Plate With Varying Concentrations of Hamster Liver Homogenate per Milliliter of S9 Mix

Dose	Concentration of S9 homogenate in the S9 mix (%)			
(μg/plate)	5	10	15	20
Trimethylolpropane triacrylate				
Solvent control[a]	14 ± 3[b]	13 ± 4	14 ± 2	5 ± 4
10	16 ± 5	20 ± 5	18 ± 2	18 ± 4
33	19 ± 6	22 ± 9	19 ± 1	18 ± 6
100	29 ± 5	27 ± 2	32 ± 5	29 ± 3
333	32 ± 9	31 ± 6	27 ± 9	36 ± 9
1,000	30 ± 2	27 ± 13	47 ± 8	49 ± 13
3,333	29 ± 3	36 ± 3	46 ± 6	38 ± 14
6,667	5 ± 4	20 ± 3	13 ± 2	15 ± 7
Positive control[c]	289 ± 14	315 ± 16	424 ± 43	441 ± 71

TABLE IV. Mutagenicity and Cytotoxicity of Acrylate Compounds in the L5178Y TK+/− Mouse Lymphoma Assay

	Dose (M)	S9	Absolute cloning efficiency[a]	Relative total growth (% of control)	Average number TFT^R colonies[b]	Mutant frequency per 10^6 survivors

Trimethylolpropane triacrylate						
Solvent control	0	−	1.00/0.87/0.71	100.0	63/62/53	70
	3×10^{-7}	−	0.70/0.74	95.5	41/48	60
	1.1×10^{-6}	−	0.75/0.88	94.5	42/61	65
	1.8×10^{-6}	−	0.80/0.59	53.5	54/50	75
	2.5×10^{-6}	−	0.43/0.31	14.5	98/112	295
	3.3×10^{-6}	−	0.34/0.37	5.0	103/111	300
Positive control	4.61×10^{-3}	−	0.39	30.0	236	610
Solvent control	0	+	0.79/0.69/0.54/0.53	100.0	53/45/48/40	78
	3.34×10^{-5}	+	0.73/0.61	102.5	61/56	85
	5.19×10^{-5}	+	0.75/0.92	97.5	57/57	70
	7.05×10^{-5}	+	0.74/0.74	64.5	68/81	100
	9.28×10^{-5}	+	0.79/0.76	32.0	80/96	115
	1.11×10^{-4}	+	0.58/0.57	8.5	107/103	180
Positive control	1.86×10^{-5}	+	0.44	31.0	161	370

TABLE V. Summary of Responses* in *S. typhimurium* and Mouse Lymphoma L5178Y TK+/−

	S. typhimurium	L5178Y TK+/−
Acrylic acid	−, −A	+, +A
Ethylene glycol diacrylate	−, −A	+, +A
Ethylene glycol dimethacrylate	−, −A	−, +A
Trimethylolpropane triacrylate	−, w+A	+, −A
Trimethylolpropane trimethacrylate	−, w+A	−, −A

*−, Negative without S9 activation; −A, negative with S9 activation; +, positive without S9 activation; +A, positive with S9 activation; w+A, weakly positive with activation.

1.1.1.3 [Anonymous (1976)]

Study reference:

Anonymous (1976). Evaluation of TMPTA, PETA and PEGDA in the Salmonella/microsome mutagenicity test.

Detailed study summary and results:

Equivalent or similar to OECD Guideline 471. No information on GLP compliance.

Test type

- Duplicate and appropriate controls included
- 4 concentrations

Test substance

- TMPTA
- Degree of purity: not stated

Administration/exposure

- *S. typhimurium* TA 1535, TA 1537, TA 98 and TA 100 (met. act.: with and without)
- Plate incorporation assay and spot test
- Type and composition of metabolic activation system:

- Prepared from 2 male rats induced with 0.1% phenobarbitone soldium in their drinking water for five days.

- Test concentrations: 0.2, 2, 20, 500 µg/plate for the incorporation assay and 5 mg for the spot test.
- Vehicle: DMSO for the spot test

Results and discussion

- Cytotoxicity: growth inhibition in the spot assay, not reported in the incorporation assay
- Negative for TA 1535, TA 1537, TA 98 and TA 100; with and without metabolic activation.
- The reversion properties of each strain are regularly checked using known mutagens.

Table 3a. Results of the spot test with TMPTA

amount TMPTA spotted in 50 µl DMSO	S-9 µl/plate	his+ revertants in each of two plates and zone of growth inhibition (cm) in brackets with: TA 1535	TA 1537	TA 98	TA 100
0 mg	50	24;27 (0)	5;7 (0)	18;18 (0)	69;70 (0)
5 mg	50	5;11 (3.5)	5;6 (2.0)	12;17 (2.5)	36;46 (3.0)
0 mg	0	59;61 (0)	4;4 (0)	27;30 (0)	110;115(0)
5 mg	0	30;34 (4.0)	3;5 (4.5)	15;17 (4.0)	55;89 (3.0)

Table 3b. Results of the plate incorporation assay with TMPTA

TMPTA µg/plate	S-9 µl/plate	his+ revertants in each of two plates with: TA 1535	TA 1537	TA 98	TA 100
0	50	4 ; 5	1 ; 6	11;18	44;55
0.2	50	2 ; 6	3 ; 5	5 ;11	50;54
2	50	8 ; 8	1 ; 9	8 ;11	43;65
20	50	5 ; 9	3 ; 4	9 ;13	54;59
500	50	4 ;13	4 ; 6	6 ; 9	55;82
0	0	1 ; 5	1 ; 4	3;10	47;57
0.2	0	7 ; 8	3 ; 3	13;17	37;43
2	0	2 ; 4	1 ; 3	12;13	34;40
20	0	4 ; 7	2 ; 6	8;16	39;49
500	0	8 ;11	2 ; 6	10;11	25;39

1.1.1.4 [NTP, 2012]

Study reference:

NTP (2012). Toxicology and carcinogenesis studies of trimethylolpropane triacrylate (technical grade) in F344/N rats and B6C3F1/N mice.

Detailed study summary and results:

Equivalent or similar to OECD Guideline 471. GLP not stated.

Test type

- Triplicate; all trials repeated
- 5 concentrations, the high dose was limited by experimental design to 10,000 µg per plate
- positive and negative control groups included

Test substance

- TMPTA
- Purity > 78%
- Batch number: 08409HI
- GC analysis indicated one major peak consisting of 87.4% of the total peak area and five impurities, each 0.1% or greater of the total peak area (0.1%, 0.1%, 9.9%, 0.2%, and 2.3%). HPLC/UV analysis indicated one major peak (78.2%) and four mpurities, each greater than 0.1% of the total peak area. hree of the four impurities were tentatively identified by HPLC with mass spectrometry detection as structurally related compounds (trimethylolpropane diacrylate, trimethylolpropane triacrylate-trimethylolpropane monoacrylate adduct, and trimethylolpropane triacrylate-trimethylol-propane diacrylate adduct); the fourth impurity was not identified. HPLC/UV analysis indicated that neither hydroquinone nor methyl hydroquinone was detected above 0.1% of the total peak area in the bulk chemical.

Administration/exposure

- Salmonella typhimurium (TA98 and TA100) or Escherichia coli (WP2 uvrA/pKM101) (met. act.: with and without)

Results and discussion

- Slight toxicity at 10,000 µg/plate with S9
- Negative for all tested strains.
- Solvent and positive control data as expected

TABLE E1
Mutagenicity of Trimethylolpropane Triacrylate in Bacterial Tester Strains[a]

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% rat S9	With 10% rat S9
TA100					
	0	111 ± 9	81 ± 5	67 ± 2	77 ± 2
	1,500	111 ± 1	75 ± 2	66 ± 5	81 ± 2
	3,000	72 ± 7	62 ± 4	60 ± 4	74 ± 7
	5,000	44 ± 2	55 ± 2	44 ± 2	83 ± 3
	7,500	32 ± 3	40 ± 4	51 ± 7	64 ± 1
	10,000	20 ± 2	35 ± 4	33 ± 2	30 ± 3
Trial summary		Negative	Negative	Negative	Negative
Positive control[b]		687 ± 13	581 ± 19	997 ± 100	740 ± 3
TA98					
	0	27 ± 3	17 ± 3	21 ± 3	27 ± 3
	1,500	22 ± 2	16 ± 2	16 ± 2	19 ± 3
	3,000	17 ± 3	17 ± 1	16 ± 2	8 ± 1
	5,000	9 ± 1[c]	12 ± 1	16 ± 1	7 ± 1
	7,500	9 ± 2[c]	7 ± 0	11 ± 1	5 ± 1
	10,000	7 ± 2[c]	3 ± 0	6 ± 1[c]	3 ± 1
Trial summary		Negative	Negative	Negative	Negative
Positive control		463 ± 19	290 ± 20	728 ± 19	624 ± 51
***Escherichia coli* WP2 *uvrA*/pKM101 (analogous to TA102)**					
	0	135 ± 4	199 ± 17	159 ± 7	129 ± 4
	1,500	110 ± 10	138 ± 4	187 ± 6	96 ± 2
	3,000	101 ± 4	115 ± 8	158 ± 10	82 ± 1
	5,000	126 ± 3	115 ± 5	160 ± 5	199 ± 9
	7,500	120 ± 3	114 ± 4	142 ± 19	118 ± 10
	10,000	121 ± 4	105 ± 6	131 ± 8	110 ± 16
Trial summary		Negative	Negative	Negative	Negative
Positive control		1,585 ± 6	1,886 ± 128	972 ± 90	912 ± 18

[a] Study was performed at SITEK Research Laboratories using the same lot (08409HI) of chemical that was used in the 2-year studies. Data are presented as revertants/plate (mean ± standard error) from three plates. 0 µg/plate was the solvent control.
[b] The positive controls in the absence of metabolic activation were sodium azide (TA100), 4-nitro-*o*-phenylenediamine (TA98), and methyl methanesulfonate (*E. coli*). The positive control for metabolic activation with all strains was 2-aminoanthracene.
[c] Slight toxicity

1.1.1.5 [Anonymous, 1979]

Study reference:

Anonymous (1979). Mutagenicity evaluation of trimethylolpropane triacrylate in the mouse lymphoma forward mutation assay.

Detailed study summary and results:

Gene mutation assay mouse lymphoma L5178Y cells. Equivalent or similar to OECD Guideline 476.

Test type

- 5 doses analysed
    - When diluted into growth medium, concentrations at 313 nl/ml and higher produced a white precipitated; lower concentrations were soluble
    - Preliminary cytotoxicity testing without activation indicated variable toxicity and complete lethality at 1.25 to 2.5 nl/ml.
- positive (ethylmethane sulfonate and dimethylnitrosamine) and negative (solvent and untreated) control groups

- 3 x 10^6 cells for each selected dose seeded in soft agar plates with selection medium and resistant (mutant) colonies are counted after 10 days incubation.

Test substance

- TMPTA
- Degree of purity: not stated

Administration/exposure

- mouse lymphoma L5178Y TK+/- cells
- Type and composition of metabolic activation system:
  - Fischer 344 male rats induced with Arochlor 1254 or other agents by injections 5 days prior to sacrifice
- Test concentrations, and reasoning for selection of doses if applicable:
  - Preliminary toxicity test: 0.004875 to 5 nL/mL (without S9); 0.004875 to 40 nL/mL (with S9).
  - Mutation test:
    - Test 1: 0.078 to 1.25 nL/mL without S9; 0.150 to 2.50 nL/mL with S9
    - Test 2: 0.150 to 1.00 nL/mL without S9; 1.250 to 10.00 nL/mL with S9
    - Test 3: 1.00 to 2.5 nL/mL without S9; 2.00 to 20 nL/mL with S9
- Vehicle: DMSO (insoluble in water)

Results and discussion

- Cytotoxicity: yes
  - After the assays were completed, the relative growth in the treated cultures were found to range from 75.2% to 3.6% without activation and from 116.3% to 4.8% with activation among all three trials.
- Genotoxic effects: Positive with and without metabolic activation; effects occurred at lower concentration in the system without activation.
  - Without activation:
    - Trial 1: mutant frequency comparable to the solvent and untreated negative control except at the high dose of 1.25 nl/ml (x3.6 times the background) (with 19.1% relative growth)
    - Trial 2: mutant frequency was about 4.2 times the background level at the high dose of 1.0 nl/ml (32.7% relative growth)
    - Trial 3: mutagenic activity at 1.0 nl/ml and 2.5 nl/ml but without activity at the intermediate doses.
  - With activation:
    - Trial 1: inconclusive (3 lowest doses only slightly toxic and no increase in mutant frequency). Contamination destroyed the results at the higher doses.

- Trial 2: no increase in mutant frequency, in spite of the high toxicity at 10.0 nl/ml (14.9% relative frenquency). Low background and positive control mutant frequencies, so the sensitivity of the assay may not have been sufficient to detect weak mutagenic activity.
- Trial 3: 6.4-fold increase in mutant frequency at 20 nl/ml (4.8% relative growth)

- Vehicle, negative and positive controls valid

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: TRIMETHYLOLPROPANE TRIACRYLATE
B. LBI CODE #: 5331
C. SOLVENT: DMSO
D. TEST DATE: 12/08/78

TEST	S-9 SOURCE	S-9 TISSUE	DAILY COUNTS (CELLS/ML X 10E5) 1	2	3	RELATIVE SUSPENSION GROWTH (% OF CONTROL)	TOTAL MUTANT CLONES	TOTAL VIABLE CLONES	RELATIVE CLONING EFFICIENCY (% OF CONTROL)	PERCENT RELATIVE GROWTH*	MUTANT FREQUENCY** (X 10E+6)
NONACTIVATION											
SOLVENT CONTROL	---	---	11.6	15.0		100.0	41.0+	256.0	100.0	100.0	16.0
SOLVENT CONTROL	---	---	13.0	14.2		100.0	32.0	237.0	100.0	100.0	13.5
UNTREATED CONTROL	---	---	12.0	16.4		109.8	30.0	177.0	71.8	78.8	16.9
EMS .5 UL/ML	---	---	9.0	8.4		42.2	952.0	82.0	33.3	14.0	1161.0
TEST COMPOUND											
0.078000 NL/ML	---	---	9.0	15.2		76.3	21.0	200.0	81.1	61.9	10.5
0.156000 NL/ML	---	---	13.0	6.6		47.9	28.0	251.0	101.8	48.7	11.2
0.313000 NL/ML	---	---	9.2	12.4		63.6	33.0	187.0	75.9	48.3	17.6
0.625000 NL/ML	---	---	14.0	12.4		96.8	45.0	190.0	77.1	74.6	23.7
1.250000 NL/ML	---	---	6.4	9.4		33.6	77.0	140.0	56.8	19.1	55.0
ACTIVATION											
SOLVENT CONTROL	RAT	LIVER	10.8	17.0		100.0	51.0+	236.0+	100.0	100.0	21.6
SOLVENT CONTROL	RAT	LIVER	11.2	17.2		100.0	75.0	161.0	100.0	100.0	46.6
UNTREATED CONTROL	RAT	LIVER	16.0	15.0		127.6	71.0+	207.0	104.3	133.0	34.3
DMN .3 UL/ML	RAT	LIVER	8.2	9.6		41.8	345.0	47.0	23.7	9.9	734.0
TEST COMPOUND											
0.156 NL/ML	RAT	LIVER	10.8	16.4		105.6	48.0+	191.0	96.2	101.6	25.1
0.313 NL/ML	RAT	LIVER	10.4	15.4		85.1	27.0	194.0	97.7	83.2	13.9
0.625 NL/ML	RAT	LIVER	10.2	14.4		78.1	79.0	236.0	118.9	92.8	33.5
1.250 NL/ML	RAT	LIVER	7.4	20.2		83.8	C	C	---	---	-----
2.500 NL/ML	RAT	LIVER	8.4	12.0		53.6	C	236.0+	118.9	63.7	

* (RELATIVE SUSPENSION GROWTH X RELATIVE CLONING EFFICIENCY) / 100
** THE RATIO OF CELLS SEEDED FOR MUTANT SELECTION TO CELLS SEEDED FOR CLONING EFFICIENCY IS 10E+4.
THEREFORE THE MUTANT FREQUENCY IS: (TOTAL MUTANT CLONES/TOTAL VIABLE CLONES)*10E-4.
THE MUTANT FREQUENCY IS GIVEN IN UNITS OF 10E-6.

+ = ONE PLATE CONTAMINATED, VALUE BASED ON REMAINING TWO PLATES.

C = CONTAMINATED (TWO OR MORE PLATES).

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: TRIMETHYLOL PROPANE TRIACRYLATE
B. LBI CODE #: 5331
C. SOLVENT: DIMETHYL SULFOXIDE
D. TEST DATE: 11/12/78

TEST	S-9 SOURCE	S-9 TISSUE	DAILY COUNTS (CELLS/ML X 10E5) 1	2	3	RELATIVE SUSPENSION GROWTH (% OF CONTROL)	TOTAL MUTANT CLONES	TOTAL VIABLE CLONES	RELATIVE CLONING EFFICIENCY (% OF CONTROL)	PERCENT RELATIVE GROWTH*	MUTANT FREQUENCY** (X 10E+6)
NONACTIVATION											
SOLVENT CONTROL	---	---	8.6	7.4		100.0	6.0	190.0	100.0	100.0	3.2
SOLVENT CONTROL	---	---	7.4	13.2		100.0	9.0	282.0	100.0	100.0	3.2
UNTREATED CONTROL	---	---	7.0	8.0		69.4	6.0	290.0	122.9	85.3	2.1
EMS .5 UL/ML	---	---	6.0	5.6		41.7	46.0	252.0	106.8	44.5	18.3
TEST COMPOUND											
0.156 NL/ML	---	---	6.2	7.8		60.0	7.0	209.0	88.6	53.1	3.3
0.313 NL/ML	---	---	9.8	5.2		63.2	5.0	281.0	119.1	75.2	1.8
0.625 NL/ML	---	---	5.6	5.8		40.3	9.0+	250.0	105.9	42.7	3.6
0.750 NL/ML	---	---	5.4	5.4		36.2	7.0	215.0	91.1	32.9	3.3
1.000 NL/ML	---	---	8.8	4.4		48.0	19.0	161.0	68.2	32.7	11.8
ACTIVATION											
SOLVENT CONTROL	RAT	LIVER	8.8	9.8		100.0	3.0	299.0	100.0	100.0	1.0
SOLVENT CONTROL	RAT	LIVER	12.6	8.4		100.0	9.0	346.0	100.0	100.0	2.6
UNTREATED CONTROL	RAT	LIVER	9.0	11.0		103.1	8.0	221.0	68.5	70.6	3.6
DMN .3 UL/ML	RAT	LIVER	4.8	5.2		26.0	75.0+	50.0	15.5	4.0	150.0
TEST COMPOUND											
1.250 NL/ML	RAT	LIVER	12.6	8.6		112.8	3.0+	218.0	67.6	76.3	1.4
2.500 NL/ML	RAT	LIVER	10.6	8.8		97.1	9.0	270.0	83.7	81.3	3.3
2.500 NL/ML	RAT	LIVER	6.6	7.8		53.6	7.0	194.0	60.2	32.2	3.6
5.009 NL/ML	RAT	LIVER	9.4	7.4		72.4	10.0	257.0	79.7	57.7	3.9
10.000 NL/ML	RAT	LIVER	3.2	6.0		18.7	8.0	256.0	79.4	14.9	3.1

* (RELATIVE SUSPENSION GROWTH X RELATIVE CLONING EFFICIENCY) / 100
** THE RATIO OF CELLS SEEDED FOR MUTANT SELECTION TO CELLS SEEDED FOR CLONING EFFICIENCY IS 10E+4.
THEREFORE THE MUTANT FREQUENCY IS: (TOTAL MUTANT CLONES/TOTAL VIABLE CLONES)*10E-4.
THE MUTANT FREQUENCY IS GIVEN IN UNITS OF 10E-6.

+ = ONE PLATE CONTAMINATED, VALUE BASED ON REMAINING TWO PLATES.

TABLE 3

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: TRIMETHYLOLPROPANE TRIACRYLATE
B. LBI CODE #: 3331
C. SOLVENT: DMSO
D. TEST DATE: 12/06/78

TEST	S-9 SOURCE	S-9 TISSUE	DAILY COUNTS (CELLS/ML X 10E5) 1	2	3	RELATIVE SUSPENSION GROWTH (% OF CONTROL)	TOTAL MUTANT CLONES	TOTAL VIABLE CLONES	RELATIVE CLONING EFFICIENCY (% OF CONTROL)	PERCENT RELATIVE GROWTH*	MUTANT FREQUENCY** (X 10E+6)
NONACTIVATION											
SOLVENT CONTROL	---	---	18.6	7.0		100.0	66.0+	170.0	100.0	100.0	38.8
SOLVENT CONTROL	---	---	18.0	8.4		100.0	22.0	156.0	100.0	100.0	14.1
UNTREATED CONTROL	---	---	14.0	10.6		105.5	92.0	227.0	139.3	146.9	40.5
EMS .5 UL/ML	---	---	12.4	5.4		47.6	412.0	67.0	41.1	19.6	614.9
TEST COMPOUND											
1.000 NL/ML	---	---	9.2	6.0		39.2	125.0+	48.0	29.4	11.6	260.4
1.250 NL/ML	---	---	6.4	5.2		23.7	84.0+	236.0+	144.8	34.2	35.6
1.500 NL/ML	---	---	3.8	6.0		12.8	C	102.0+	62.6	8.0	-----
2.000 NL/ML	---	---	4.6	3.6		11.8	74.0+	107.0	65.6	7.7	69.2
2.500 NL/ML	---	---	1.8	9.2		19.6	60.0	30.0	18.4	3.6	200.0
ACTIVATION											
SOLVENT CONTROL	RAT	LIVER	12.4	6.8		100.0	38.0	244.0	100.0	100.0	15.6
SOLVENT CONTROL	RAT	LIVER	12.4	6.6		100.0	20.0+	172.0	100.0	100.0	11.6
UNTREATED CONTROL	RAT	LIVER	9.6	10.6		122.5	33.0	221.0+	106.3	130.1	14.9
DMN .3 UL/ML	RAT	LIVER	9.2	4.6		50.9	108.0+	26.0+	12.5	6.4	415.4
TEST COMPOUND											
2.000 NL/ML	RAT	LIVER	8.8	10.0		105.9	42.0+	183.0	88.0	93.2	23.0
2.500 NL/ML	RAT	LIVER	10.4	9.2		115.2	29.0	210.0+	101.0	116.3	13.8
5.000 NL/ML	RAT	LIVER	6.4	8.8		67.8	44.0	236.0	113.5	76.9	18.6
10.000 NL/ML	RAT	LIVER	10.0	10.0		120.4	47.0+	167.0+	80.3	96.6	28.1
20.000 NL/ML	RAT	LIVER	1.4	3.2		11.6	77.0+	87.0	41.8	4.8	88.5

* (RELATIVE SUSPENSION GROWTH X RELATIVE CLONING EFFICIENCY) / 100
** THE RATIO OF CELLS SEEDED FOR MUTANT SELECTION TO CELLS SEEDED FOR CLONING EFFICIENCY IS 10E+4. THEREFORE THE MUTANT FREQUENCY IS: (TOTAL MUTANT CLONES/TOTAL VIABLE CLONES)*10E-4. THE MUTANT FREQUENCY IS GIVEN IN UNITS OF 10E-6.

+ = ONE PLATE CONTAMINATED, VALUE BASED ON REMAINING TWO PLATES.

C = CONTAMINATED (TWO OR MORE PLATES).

1.1.1.6 [Moore et al., 1989]

Study reference:

Moore MM, Harrington-Brock K, Doerr CL, Dearfield KL (1989). Differential mutant quantitation at the mouse lymphoma tk and CHO hgprt loci. Mutagenesis vol. 4 no 5 pp.394-403.

Detailed study summary and results:

Gene mutation assay & mouse lymphoma assay: Equivalent or similar to OECD Guideline 476

Chromosomal aberrations assay: Equivalent or similar to OECD Guideline 473

GLP not stated

Test type

- Duplicate
- 3 doses
- positive and negative control groups included
- Mutagenicity experiment: CHO cells: cells were treated for 4 hours. $3x10^6$ cells were cloned on day 7 using 6-thioguanine for HGPRT mutant selection. Cells (600) cloned in the absence of 6-thioguanine were used for viable count determination. After a 12-13 day incubation period, colonies were counted on an Artek model 880 automatic colony counter.

- Mouse lymphoma assay (both cytogenetic and mutant analysis): cells were treated for 4 hours. Following treatment, cells for mutant frequency analysis were centrifuged, washed twice with fresh medium and resuspended for incubation. Cells were growth for a 2-day expression period and then cloned with TFT selection and without selection. After 9-11 days of incubation, colonies were counted on an Artek model 880 automatic colony counter. A separate small-colony TK and large colony TK mutant frequency was calculated.
- Cytogenetic analysis: following the 4h treatment, cells were washed and incubated with BrdUrd from 15-16h. For each test concentration, 100 well-spread, complete appearing, first-division metaphases were analysed.

Test substance

- TMPTA
- Degree of purity: not stated

Administration/exposure

- Chinese hamster Ovary (CHO) (met. act.: without); strain K1-BH4
- Mouse lymphoma TK+/- cells
- Test concentrations:
  - 0, 0.2, 0.6, 0.7 µg/mL for 4 hours (CHO assay)
  - 0, 0.6, 0.65, 0.7 µg/mL (mouse lymphoma L5178Y cells)
- Vehicle: DMSO

Results and discussion

- Cytotoxicity : yes (see table below on survival)
- Genotoxic effects:
  - Negative for gene mutations in CHO
  - Positive for chromosome aberrations in CHO cells
  - Positive (exclusive induction of small colonies) for mouse lymphoma L5178Y cells
- Concurrent negative (vehicle) and positive control data: valid

Table I. Gross chromosome aberration analysis and mutant frequency in mouse lymphoma and CHO cells

Mutagen/cell line	Dose (µg/ml)	Chromatid		Chromosome		≥ 10	Total number of ab[a]	Cells w/ab	M.F.[b] × 10^{-6} s/l	Survival[c] %
		Breaks	Rearrangements	Breaks	Rearrangements					

Trimethylolpropane triacrylate/mouse lymphoma	0.0	0	1	1	1	0	3	3	37/29	100
	0.6	6	1	1	0	0	8	5	119/24	51
	0.65	4	6	1	0	0	11	11	151/38	40
	0.7	7	8	4	2	0	21	14	232/51	28
Trimethylolpropane triacrylate/CHO	0.0	0	0	1	0	0	1	1	3	100
	0.2	3	1	9	0	0	13	10	9	72
	0.6	10	18	5	1	0	34	23	8	22
	0.7	7	22	7	1	0	37	26	1	13

[a]ab, aberrations. One hundred cells were scored for each data point except for the 37.5 μg/ml dose of ethyl acrylate, in which only 50 cells could be analyzed. The numbers in the table for this culture are shown as double the actual counts.
[b]M.F., mutant frequency. The TK mutant frequency in mouse lymphoma cells is presented as small/large colony frequency (s/l); the HGPRT mutant frequency in CHO cells is presented as the total mutant frequency. For each cell type, the aberration analysis and mutant frequency analysis was performed on duplicate cultures in the same experiment.
[c]Survival is calculated according to the method of Clive and Spector (1975).
[d]Colony sizing was not performed. Total mutant frequency is presented.

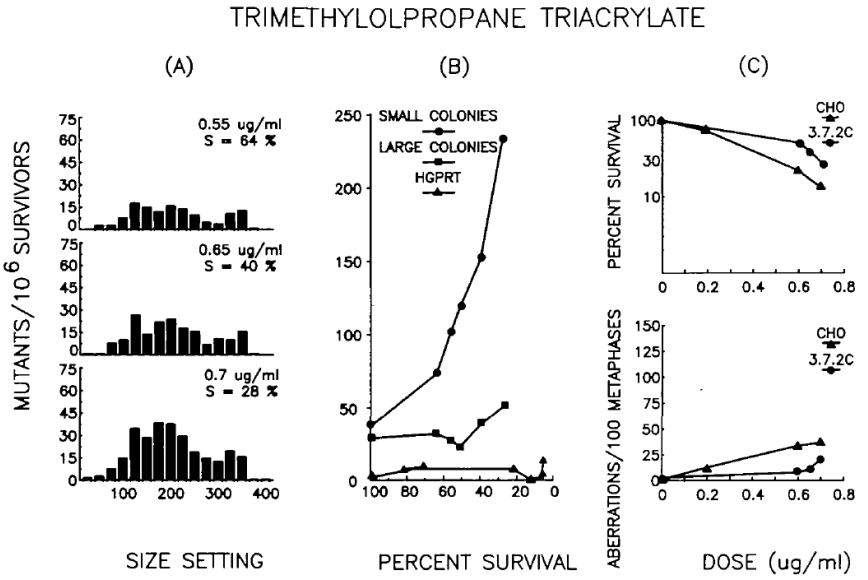

Fig. 6. Genotoxicity of trimethylolpropane triacrylate. **(A)** Colony sizing curves for mouse lymphoma cells treated by three concentrations of trimethylolpropane triacrylate. **(B)** Small- and large-colony TK mutant frequency for treated mouse lymphoma cells and HGPRT mutant frequency for treated CHO cells. **(C)** Chromosome aberration analysis for treated mouse lymphoma and CHO cells.

1.1.1.7 [Anonymous, 2005]

Study reference:

Anonymous (2005). In Vitro Mammalian Chromosome Aberration Test in Cultured Human Lymphocytes.

Detailed study summary and results:

Mammalian chromosome aberration test

OECD Guideline 473 ; EU Method B.10 ; EPA OPPTS 870.5375

GLP compliant

Test type

- 3 independent experiments with the initial experiment considered as a “preliminary” experiment that resulted in cytotoxicity at all concentrations tested and no further evaluations were conducted.
- In the first experiment, lymphocyte cultures were exposed to the test or control items (with or without S9 mix) for 3 hours then rinsed. In the second experiment, cells were exposed under the

same regimen as for the first experiment, both with and without S9. Cells were harvested 20 hours after the beginning of treatment, corresponding to approximately 1.5 normal cell cycles.

- positive (mitomycin C 3 µg/ml and cyclophosphamide 12.5 or 25 µg/ml) and negative control groups included
- Analysis of 100 metaphases/culture whenever possible; Only 50 metaphases/culture were analyzed when at least 10% of the cells with structural chromosome aberrations were observed.

Test substance

- TMPTA
- Degree of purity = 84.6%
- Lot No.: 00885GD4

Administration/exposure

- Lymphocytes: primary cell cultures from human peripheral blood (met. act.: with and without)
- Type and composition of metabolic activation system: The S9 mix consists of induced enzymatic systems contained in rat liver post-mitochondrial fraction (S9 fraction) and the cofactors necessary for their function. S9 fraction was purchased from Moltox (Molecular Toxicology, INC, Boone, NC 28607, USA) and obtained from the liver of rats treated with Aroclor 1254 (500 mg/kg) by the intraperitoneal route.
- Test concentrations:
    - Preliminary experiment: 23.1, 45.7, 92.6, 185, 370, 740, 1480 and 2960 µg/mL for the preliminary experiment both with and without S9 mix
    - Main experiment:
    - 1) 0.78, 1.56, 3.13, 6.25, 12.5, 18.75, 25 and 37.5 µg/mL for the first experiment without S9 mix → metaphase analysis for 3.13, 6.25, 18.75 µg/ml
    - 2) 1.56, 3.13, 6.25, 12.5, 18.75, 25, 37.5 and 50 µg/mL for the first experiment with S9 mix → metaphase analysis for 18.75, 37.5 and 50 µg/mL
    - 3) 3.13, 6.25, 9.38, 12.5, 18.75 and 28.13 µg/mL, for the second experiment without S9 mix → metaphase analysis for 9.38, 12.5, 18.75 µg/ml
    - 4) 9.38, 18.75, 28.13, 37.5, 50 and 75 µg/mL, for the second experiment with S9 mix → metaphase analysis for 28.13, 37.5 and 50 µg/mL
- Vehicle: DMSO (freely soluble)
- Statistical methods: For each test and for each harvest time, the frequency of cells with structural chromosome aberrations (excluding gaps) in treated cultures was compared to that of the vehicle control cultures. If necessary, the results were compared using the $\chi 2$ test, in which p = 0.05 was used as the lowest level of significance.

Results and discussion

- Concentrations in the first experiment selected based on the preliminary experiment. For the second experiment, any toxicity indicated by the reduction of mitotic index (MI) in the first experiment was also taken into account.
- No precipitation and no emulsion at the end of the treatment period at the concentrations tested
- Cytotoxicity:
    - Without S9: cytotoxicity observed at all concentrations of the preliminary experiment (90% for the lowest concentration of 23.1 µg/ml and 100% for other concentrations) and in both first and second experiments at conc. ≥ 12.5 µg/mL (ranging between 45-100% cytotoxicity and between 26-83% cytotoxicity, respectively)
    - With S9: cytotoxicity between 50-100% in the preliminary experiment; between 36-52% in the first experiment at concentrations ≥ 25 µg/ml and between 37-98% in the second experiment at concentrations ≥ 37.5 µg/ml
- Genotoxic effects : Positive with and without metabolic activation
    - Without S9 mix:
        - First experiment: statistically significant increase in the frequency of cells with structural chromosomal aberrations was noted at 18.75 µg/mL (46% versus 1.5% for the vehicle control).
        - Second experiment: statistically significant and concentration-related increases in the frequency of cells with structural chromosomal aberrations were noted at concentrations ≥ 9.38 µg/mL (6, 10.7 and 30% at concentrations of 9.38, 12.5, and 18.75 µg/mL, respectively, versus 0.5% for the vehicle control).
    - With S9 mix:
        - First experiment: statistically significant and concentration-related increases in the frequency of cells with structural chromosomal aberrations were noted at the concentrations of 37.5 and 50 µg/mL (21 and 53%, respectively versus 1% for the vehicle control).
        - Second experiment: statistically significant increases in the frequency of cells with structural chromosomal aberrations, without any clear evidence of a concentration-relationship, were noted at concentrations of 28.13 and 50 µg/mL (4.5% and 19%, respectively, versus 1% for the vehicle control).
- Vehicle and positive controls valid

Table 1: First experiment without S9 mix: mitotic index (3-hour treatment, 20-hour harvest)

Concentrations µg/mL	Slide Nb.	Mitotic index (%)	Mean	% of the control
0	49 M	7.2	5.45	100
	66 F	3.7		
0.78	54 M	8.1	6.10	112
	74 F	4.1		
1.56	73 M	5.2	5.05	93
	43 F	4.9		
3.13	64 M	5.8	5.45	100
	78 F	5.1		
6.25	50 M	5.6	4.45	82
	57 F	3.3		
12.5	75 M	2.8	3.00	55
	81 F	3.2		
18.75	65 M	3.4	3.00	55
	70 F	2.6		
25	79 M	1.2	0.60	11
	52 F	0.0		
37.5	45 M	0.0	0.00	0
	83 F	0.0		
MMC 3 µg/mL	56 M	2.7	2.85	52
	61 F	3.0		

M: male
F: female
0: vehicle control (DMSO)
MMC: mitomycin C
Nb.: number

Table 2: First experiment without S9 mix: chromosome aberrations (3-hour treatment, 20-hour harvest)

Concentrations µg/mL	Slide Nb.	Nb. of cells scored	NA	Structural chromosome aberrations (type and number) G	Chromatid D	Chromatid Exch	Chromosome D	Chromosome Exch	MA	PU	Total +G	Total -G	Cells with structural chromosome aberrations Nb. +G	mean % +G	Nb. -G	mean % -G
0	49 M	100	0	1	1	0	0	0	0	0	**4**	**3**	2	**2.0**	1	**1.5**
	66 F	100	1	0	2	0	0	0	0	0			2		2	
3.13	64 M	100	1	0	1	0	0	0	0	0	**3**	**2**	1	**1.5**	1	**1.0**
	78 F	100	1	1	1	0	0	0	0	0			2		1	
6.25	50 M	100	0	0	0	0	1	0	0	0	**7**	**6**	1	**3.0**	1	**2.5**
	57 F	100	1	1	3	1	1	0	0	0			5		4	
18.75	65 M	50	0	5	28	1	2	0	3	0	**95**	**84**	22	**50.0**	19	**46.0**
	70 F	50	1	6	40	6	1	0	3	0			28		27	***
MMC	56 M	50	0	1	39	9	2	0	5	0	**100**	**99**	27	**54.0**	27	**54.0**
3 µg/mL	61 F	50	2	0	30	10	2	0	2	0			27		27	***

NA: numerical aberrations, G: gap, D: deletion, Exch: exchange, MA: multiple aberrations, PU: pulverization.
M: male
F: female
0: vehicle control (DMSO)
MMC: mitomycin C
Statistical analysis: X2 test ***: $p < 0.001$ (performed only for cells with structural aberrations excluding gaps)
Nb.: number

Table 3: Second experiment without S9 mix: mitotic index (3-hour treatment, 20-hour harvest)

Concentrations µg/mL	Slide Nb.	Mitotic index (%)	Mean	% of the control
0	101 M	5.3	5.60	100
	85 F	5.9		
3.13	111 M	7.4	7.45	133
	98 F	7.5		
6.25	90 M	3.5	5.90	105
	109 F	8.3		
9.38	104 M	6.9	5.55	99
	117 F	4.2		
12.5	92 M	3.3	4.00	71
	112 F	4.7		
18.75	108 M	3.4	4.15	74
	87 F	4.9		
28.13	100 M	1.9	0.95	17
	106 F	0.0		
MMC 3 µg/mL	89 M	2.3	2.65	47
	97 F	3.0		

M: male
F: female
0: vehicle control (DMSO)
MMC: mitomycin C
Nb.: number

Table 4: Second experiment without S9 mix: chromosome aberrations (3-hour treatment, 20-hour harvest)

Concentrations µg/mL	Slide Nb.	Nb. of cells scored	NA	Structural chromosome aberrations (type and number) G	Chromatid D	Chromatid Exch	Chromosome D	Chromosome Exch	MA	PU	Total +G	Total -G	Cells with structural chromosome aberrations Nb. +G	mean %	Nb. -G	mean %
0	101 M	100	1	0	1	0	0	0	0	0	**2**	**1**	1	**1.0**	1	**0.5**
	85 F	100	0	1	0	0	0	0	0	0			1		0	
9.38	104 M	100	0	0	5	0	0	0	0	0	**15**	**15**	5	**6.0**	5	**6.0**
	117 F	100	1	0	8	0	2	0	0	0			7		7	**
12.5	92 M	50	0	3	6	0	0	0	1	0	**25**	**20**	7	**12.7**	6	**10.7**
	112 F	100	0	2	8	2	1	0	2	0			12		10	***
18.75	108 M	50	1	1	16	2	2	0	3	0	**60**	**53**	14	**32.0**	13	**30.0**
	87 F	50	0	6	24	4	1	0	1	0			18		17	***
MMC	89 M	50	0	3	27	8	6	0	2	0	**98**	**94**	29	**57.0**	29	**57.0**
3,0 µg/mL	97 F	50	0	1	34	11	4	0	2	0			28		28	***

NA: numerical aberrations, G: gap, D: deletion, Exch: exchange, MA: multiple aberrations, PU: pulverization.
M: male
F: female
0: vehicle control (DMSO)
MMC: mitomycin C
Statistical analysis: X2 test
**: $p<0.01$ (performed only for cells with structural aberrations excluding gaps)
***: $p < 0.001$
Nb.: number

Table 5: First experiment with S9 mix: mitotic index (3-hour treatment, 20-hour harvest)

Concentrations µg/mL	Slide Nb.	Mitotic index (%)	Mean	% of the control
0	58 M	5.5	6.00	100
	47 F	6.5		
1.56	82 M	4.7	4.90	82
	69 F	5.1		
3.13	67 M	4.4	4.35	73
	53 F	4.3		
6.25	48 M	6.2	5.15	86
	63 F	4.1		
12.5	59 M	4.7	5.20	87
	71 F	5.7		
18.75	44 M	7.2	6.05	101
	77 F	4.9		
25	84 M	4.0	3.85	64
	51 F	3.7		
37.5	76 M	2.9	2.90	48
	80 F	2.9		
50	60 M	5.4	3.75	63
	55 F	2.1		
CPA 12.5 µg/mL	68 M	3.3	3.50	58
	72 F	3.7		
CPA 25 µg/mL	46 M	3.9	3.10	52
	62 F	2.3		

M: male
F: female
0: vehicle control (DMSO)
CPA: cyclophosphamide
Nb.: number

Table 6: First experiment with S9 mix: chromosome aberrations (3-hour treatment, 20-hour harvest)

Concentrations µg/mL	Slide Nb.	Nb. of cells scored	NA	Structural chromosome aberrations (type and number) G	Chromatid D	Chromatid Exch	Chromosome D	Chromosome Exch	MA	PU	Total +G	Total -G	Cells with structural chromosome aberrations Nb. +G	mean %	Nb. -G	mean %
0	58 M	100	0	0	1	0	0	0	0	0	**2**	**2**	1	**1.0**	1	**1.0**
	47 F	100	1	0	1	0	0	0	0	0			1		1	
18.75	44 M	100	0	0	3	0	0	0	0	0	**8**	**7**	3	**3.0**	3	**2.5**
	77 F	100	0	1	4	0	0	0	0	0			3		2	
37.5	76 M	50	0	1	14	1	0	0	1	0	**35**	**32**	10	**22.0**	10	**21.0**
	80 F	50	0	2	16	0	0	0	0	0			12		11	***
50	60 M	50	0	8	44	4	0	0	6	0	**119**	**108**	27	**54.0**	27	**53.0**
	55 F	50	0	3	43	4	3	0	4	0			27		26	***
CPA	68 M	50	0	1	26	1	4	0	1	0	**57**	**53**	19	**33.0**	17	**30.0**
12.5 µg/mL	72 F	50	0	3	15	3	3	0	0	0			14		13	***

NA: numerical aberrations, G: gap, D: deletion, Exch: exchange, MA: multiple aberrations, PU: pulverization.
M: male
F: female
0: vehicle control (DMSO)
CPA: cyclophosphamide
Statistical analysis: X2 test ***: $p < 0.001$ (performed only for cells with structural aberrations excluding gaps)
Nb.: number

Table 7: Second experiment with S9 mix: mitotic index (3-hour treatment, 20-hour harvest)

Concentrations µg/mL	Slide Nb.	Mitotic index (%)	Mean	% of the control
0	91 M	5.4	5.60	100
	103 F	5.8		
9.38	113 M	4.2	4.75	85
	94 F	5.3		
18.75	102 M	5.3	5.40	96
	110 F	5.5		
28.13	86 M	6.7	6.35	113
	99 F	6.0		
37.5	118 M	2.8	3.05	54
	93 F	3.3		
50	114 M	3.7	3.50	63
	107 F	3.3		
75	95 M	0.0	0.10	2
	116 F	0.2		
CPA 12.5 µg/mL	88 M	2.0	2.95	53
	105 F	3.9		
CPA 25 µg/mL	115 M	1.5	2.80	50
	96 F	4.1		

M: male
F: female
0: vehicle control (DMSO)
CPA: cyclophosphamide
Nb.: number

Table 8: Second experiment with S9 mix: chromosome aberrations (3-hour treatment, 20-hour harvest)

Concentrations µg/mL	Slide Nb.	Nb. of cells scored	NA	Structural chromosome aberrations (type and number)									Cells with structural chromosome aberrations			
				G	Chromatid		Chromosome		MA	PU	Total +G	Total -G	Nb. +G	mean %	Nb. -G	mean %
					D	Exch	D	Exch								
0	91 M	100	0	0	1	0	0	0	0	0	**2**	**2**	1	**1.0**	1	**1.0**
	103 F	100	0	0	1	0	0	0	0	0			1		1	
28.13	86 M	100	3	1	12	0	0	0	0	0	**16**	**13**	8	**5.5**	8	**4.5**
	99 F	100	0	2	1	0	0	0	0	0			3		1	*
37.5	118 M	100	0	0	6	0	0	0	0	0	**8**	**6**	3	**2.5**	3	**1.5**
	93 F	100	2	2	0	0	0	0	0	0			2		0	
50	114 M	50	0	5	14	1	0	0	1	0	**34**	**29**	16	**23.0**	12	**19.0**
	107 F	50	1	0	11	0	2	0	0	0			7		7	***
CPA	88 M	50	0	2	17	2	1	0	0	0	**46**	**43**	15	**31.0**	14	**30.0**
12.5 µg/mL	105 F	50	1	1	20	1	1	0	1	0			16		16	***

NA: numerical aberrations, G: gap, D: deletion, Exch: exchange, MA: multiple aberrations, PU: pulverization.
M: male
F: female
0: vehicle control (DMSO)
CPA: cyclophosphamide
Statistical analysis: X2 test *: $p < 0.05$ ***: $p < 0.001$ (performed only for cells with structural aberrations excluding gaps)
Nb.: number

From January 2003 to September 2003

Historical data: experiments with S9 mix
Chromosomal aberrations on human lymphocytes

	Vehicle control [a]				CPA 25 [a]		CPA 12,5 [a]	
	20 hours [b]		44 hours [b]		20 hours [b]		20 hours [b]	
study No.	MI	% ab cells	MI	% ab cells	MI	% ab. cells	MI	% ab. cells
1	6,9	2,5	-	-	2,3	48,0	-	-
	2,6	1,5	5,2	0,5	1,5	32,0	-	-
2	4,1	0,0	-	-	-	-	2,2	19,0
	7,6	0,5	5,1	0,0	2,6	23,0	-	-
3	3,7	2,0	-	-	1,3	36,0	-	-
	4,7	2,5	6,2	0,0	-	-	1,7	24,0
4	2,7	1,0	-	-	-	-	1,2	34,0
	5,7	0,5	5,6	0,5	-	-	2,6	18,0
5	8,7	1,0	-	-	-	-	4,0	22,0
	4,1	0,0	5,0	0,5	-	-	3,0	15,0
6	5,0	1,0	-	-	-	-	3,1	30,0
	5,2	1,0	4,5	0,5	-	-	4,4	16,0
7	5,9	0,5	-	-	-	-	3,1	28,0
	3,4	0,5	3,0	0,0	1,3	23,0	-	-
8	6,2	0,5	-	-	-	-	4,4	24,8
	3,5	0,5	4,5	0,0	-	-	3,4	26,0
9	4,2	0,0	-	-	-	-	2,6	23,0
	3,4	1,0	2,9	0,0	1,2	27,0	-	-
10	3,9	0,5	-	-	-	-	1,3	24,0
	6,2	1,0	2,9	0,0	-	-	3,3	31,0
11	4,5	1,0	-	-	-	-	2,0	29,0
	3,5	1,0	1,8	1,0	1,2	27,1	-	-
12	4,4	1,0	-	-	1,7	23,0	-	-
	6,2	1,0	4,7	0,5	3,6	29,0	-	-
minimum	**2,6**	**0,0**	**1,8**	**0,0**	**1,2**	**23,0**	**1,2**	**15,0**
maximum	**8,7**	**2,5**	**6,2**	**1,0**	**3,6**	**48,0**	**4,4**	**34,0**

MI: mitotic index
% ab. cells: frequency of aberrant cells expressed in term of gap excluded
CPA: cyclophosphamide (µg/mL)
[a]: cultures treated with the vehicle or positive controls for 3 hours
[b]: harvest time after the beginning of treatment
-: not done

1.1.2 Animal data

1.1.2.1 [Anonymous, 2006]

Study reference:

Anonymous (2006). Bone marrow micronucleus test by oral route in mice.

Detailed study summary and results:

Test type

Micronucleus assay (chromosome aberration)

OECD Guideline 474

EU Method B.12

EPA OPPTS 870.5395

GLP compliant

Test substance

- TMPTA
- Degree of purity = 87.9%
- Lot No.: 07415FZ4

Test animals

- mouse (Swiss Ico: OF1 (IOPS Caw)) male/female
- 5/sex/dose
- Approximately 6 weeks old on the day of treatment. Weight: at the beginning of treatment the mean body weight was 32.3 g for males (ranging from 28.4 to 35.2 g) and 23.9 g for females (ranging from 21.0 to 26.5 g). The body weight of each animal was within ± 20% of the mean body weight per sex

Administration/exposure

- 437.5, 875 and 1750 mg/kg bw (for males) or 500, 1000 and 2000 mg/kg bw (for females) (nominal conc.) based on preliminary toxicity assay
- Vehicle: corn oil
- Single administration by gavage
- Animals killed 24 (control, low, intermediate and high dose groups) or 48 hours (control and high dose groups) after treatment. The animals of the positive control group were killed 24 hours after treatment. Bone marrow smears were then prepared.
- Positive (cyclophosphamide once by the oral route at a dose of 50 mg/kg) and negative (vehicle) controls included
- For each animal, the number of the micronucleated polychromatic erythrocytes (MPE) was counted in 2000 polychromatic erythrocytes. The polychromatic (PE) and normochromatic (NE) erythrocyte ratio was established by scoring a total of 1000 erythrocytes (PE + NE).
- Statistical methods: Normality and homogeneity of variances was tested using a Kolmogorov Smirnov test and a Bartlett test. If normality and homogeneity of variances were demonstrated, the statistical comparisons was performed using a Student t-test (2 groups) or a one-way analysis of variance (≥ 3 groups) followed by a Dunnett test (if necessary). If normality or homogeneity of variances was not demonstrated, a Mann/Whitney test (2 groups) or a Kruskall Wallis test (≥ 3 groups) was performed followed by a Dunn test (if necessary). All these analyses were performed using the software SAS Enterprise Guide V2 (2.0.0.417, SAS Institute Inc), with a level of significance of 0.05 for all tests.

Results and discussion

- Toxicity:
    - Preliminary toxicity study at 1750 and 2000 mg/kg: males were the more sensitive sex. At 2000 mg/kg, neither mortality nor clinical signs were noted in the 3 treated females. In males, 1/3 males was found dead 24 hours following treatment. At the same time and until sacrifice, piloerection and ocular secretion or soiled urogenital area were noted in the two surviving males. A confirmatory assay was performed using 3 males at 2000 mg/kg in order to confirm the previous findings. Two males were found dead 24 and 48 hours following treatment. The surviving male showed piloerection. Based on these results, an additional test was performed using 3 males at 1750 mg/kg. Only piloerection was noted in males given 1750 mg/kg in this second preliminary test and no mortality was induced.
    - Main test in males: piloerection at 875 mg/kg bw 24 hours following the administration of the test item; 2 deaths 24h following the administration and at the same time piloerection in surviving animal at 1750 mg/kg bw
    - Main test in females: no clinical signs and no mortality attributed to the treatment with the test item were observed in animals given 0, 500, 1000 or 2000 mg/kg.
    - Accidental deaths due to dosing errors occurred in both males and females as follows: one male in the vehicle control group, one male at 437.5 mg/kg and four females at 2000 mg/kg. Since three of the four females from the high dose group could be replaced by three supplementary animals, raw data for micronucleus analysis were missing for two males (one from the vehicle control group, 48-hour harvest time and one from the 437.5 mg/kg group) and one female (from the 2000 mg/kg group, 48 hour harvest time).
- For both males and females, the mean values of MPE as well as the PE/NE ratios in the groups treated with the test item, were equivalent to those of the vehicle control group, for both harvest times.
- Genotoxic effects: Negative (male/female)
- Vehicle and positive controls valid

Group	Doses (mg/kg)	MPE/1000PE mean	(sd)		PE/NE ratio mean	(sd)	Time of sacrifice after the last administration
Males							
Vehicle	-	1.5	(1.6)		0.7	(0.1)	
Test item	437.5	1.1	(0.5)		0.5	(0.1)	24 h
	875	0.7	(0.8)		0.4	(0.3)	
	1750	1.9	(1.2)		0.8	(0.3)	
Cyclophosphamide	50	23.9	(11.0)	**	0.7	(0.1)	
Females							
Vehicle	-	1.2	(0.4)		0.9	(0.3)	
Test item	500	0.8	(0.4)		0.8	(0.2)	
	1000	0.8	(0.7)		1.0	(0.4)	24 h
	2000	0.9	(1.1)		1.0	(0.4)	
Cyclophosphamide	50	13.6	(7.7)	*	0.7	(0.3)	
Males							
Vehicle	-	0.6	(0.5)		0.4	(0.1)	48 h
Test item	1750	0.8	(0.9)		0.5	(0.2)	
Females							
Vehicle	-	0.5	(0.4)		0.9	(0.1)	48 h
Test item	2000	1.1	(0.9)		0.7	(0.2)	

Five animals per group except for the following groups which contained four animals:
Males at 0 mg/kg (48 hours after treatment) and 437.5 mg/kg (24 hours after treatment) and
Females at 2000 mg/kg (48 hours after treatment)
MPE: Micronucleated Polychromatic Erythrocytes
PE: Polychromatic Erythrocytes
NE: Normochromatic Erythrocytes
sd: standard deviation

Vehicle and test item:
Number of administrations: one
Route: Oral
Vehicle: Corn oil

Cyclophosphamide:
Number of administrations: one
Route: oral
Vehicle: water

Statistical significance: *: $p < 0.05$ **: $p < 0.01$

1.1.2.2 [NTP, 2005]

Study reference:

NTP (2005). NTP report on the toxicology studies of trimethylolpropane triacrylate (technical grade) (CAS No. 15625-89-5) in F344/N rats, B6C3F1 mice and genetically modified (FVB Tg. Ac hemizygous) mice (dermal studies).

Detailed study summary and results:

Test type

micronucleus assay (chromosome aberration)
Not guideline

Test substance

- TMPTA
- Degree of purity = 80%

- GC indicated one major peak and two impurities with areas of 6.5% and 3.4% relative to the major peak area. HPLC indicated a major peak and five impurities with a combined area of 22.2%. HPLC/MS indicated 10 impurities contributing or corresponding to the impurity peaks identified by HPLC. These impurities included four structurally related acrylates or adducts: trimethylolpropane diacrylate, trimethylolpropane triacrylate acrylic acid adduct, trimethylolpropane triacrylate-trimethylolpropane monoacrylate adduct, and trimethylolpropane triacrylatetrimethylolpropane diacrylate adduct. No substantial amount of 4-methoxyphenol, a stabilizer added to trimethylolpropane triacrylate, was detected.
- Batch number: 01031AW

Test animals

- mouse (B6C3F) for the 14 week study (10/sex)
- Genetically modified (FVB tg.AC hemizygous) mice for the 6 month study (15/sex)
- Age at the study initiation: 6 weeks

Administration/exposure

- The study was performed as part of subchronic dermal studies. Male and female mice were dermally exposed to the test substance 5 times per week for 14 or 28 weeks. Blood was collected from the retroorbital sinus and stained for analysis of micronuclei and NCE/PCE ratio at the end of exposure.
- Slides were scanned to determine the frequency of micronuclei in 2,000 normochromatic erythrocytes (NCEs) in each of 10 (3-month study) or up to 15 (6-month study) animals per dose group. In addition, the percentage of normochromatic erythrocytes in 1,000 total erythrocytes per animal was determined to provide a measure of chemical-induced bone marrow toxicity.
- 0, 0.75, 1.5, 3, 6, 12 mg/kg (nominal conc.)
- Vehicle: acetone
- Dermal exposure; 5/weeks for 14 or 28 weeks
- Statistical methods: The results were tabulated as the mean of the pooled results from all animals within a treatment group plus or minus the standard error of the mean. The frequency of micronucleated cells among NCEs was analyzed by a statistical software package that tested for increasing trend over dose groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each dosed group and the control group (ILS, 1990). In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single dosed group is less than or equal to 0.025 divided by the number of dose groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials. Results of the 3- and 6-month studies were accepted without repeat tests, because additional test data could not be obtained. Ultimately, the final call is determined by the

scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects.

Results and discussion

- Toxicity: no toxicity on the bone marrow in the 3-month study (PCE/NCE ratio unaltered); decrease in the percentage of NCEs among total erythrocytes in the 6-month study at 12 mg/kg in both males and females
- Genotoxic effects: Negative (male/female)
- Vehicle controls valid: yes; positive control not included

Frequency of Micronuclei in Peripheral Blood Normochromatic Erythrocytes of B6C3F$_1$ Mice Following Dermal Application of Trimethylolpropane Triacrylate for 3 Months[a]

Compound	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[c]	NCEs[b] (%)
Male					
Acetone[d]		10	1.25 ± 0.27		98.0 ± 0.1
Trimethylolpropane triacrylate	0.75	10	1.55 ± 0.17	0.2112	97.4 ± 0.2
	1.5	10	0.95 ± 0.19	0.8173	97.8 ± 0.2
	3	10	1.20 ± 0.31	0.5568	97.5 ± 0.2
	6	10	1.10 ± 0.19	0.6693	98.0 ± 0.2
	12	10	0.60 ± 0.10	0.9837	97.8 ± 0.1
			P=0.993[e]		
Female					
Acetone		10	0.60 ± 0.15		97.9 ± 0.2
Trimethylolpropane triacrylate	0.75	10	0.50 ± 0.17	0.6651	98.0 ± 0.2
	1.5	10	0.85 ± 0.18	0.1765	98.3 ± 0.1
	3	10	0.70 ± 0.21	0.3474	98.1 ± 0.1
	6	10	0.65 ± 0.22	0.4207	97.8 ± 0.2
	12	10	0.80 ± 0.17	0.2248	97.9 ± 0.2
			P=0.232		

[a] Study was performed at SITEK Research Laboratories, Inc. The detailed protocol is presented by MacGregor *et al.* (1990).
[b] NCE=normochromatic erythrocyte
[c] Mean ± standard error
[d] Pairwise comparison with the controls, significant at P≤0.005 (ILS, 1990)
[e] Vehicle control
Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test, significant at P≤0.025 (ILS, 1990)

TABLE C2
Frequency of Micronuclei in Peripheral Blood Normochromatic Erythrocytes of Tg.AC Hemizygous Mice Following Dermal Application of Trimethylolpropane Triacrylate for 6 Months[a]

Compound	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[c]	NCEs[b] (%)
Male					
Acetone[d]		14	2.82 ± 0.28		97.4 ± 0.1
Trimethylolpropane triacrylate	0.75	15	3.23 ± 0.55	0.1837	97.3 ± 0.2
	1.5	12	3.17 ± 0.35	0.2358	97.2 ± 0.1
	3	14	2.11 ± 0.17	0.9559	97.5 ± 0.1
	6	13	1.88 ± 0.30	0.9874	97.2 ± 0.2
	12	11	2.36 ± 0.24	0.8399	93.7 ± 1.2
			P=0.990[e]		
Female					
Acetone		15	1.00 ± 0.18		97.6 ± 0.1
Trimethylolpropane triacrylate	0.75	14	1.32 ± 0.18	0.1274	97.4 ± 0.1
	1.5	12	1.25 ± 0.22	0.1931	97.8 ± 0.1
	3	14	1.21 ± 0.21	0.2187	97.5 ± 0.3
	6	14	1.14 ± 0.16	0.2994	97.7 ± 0.1
	12	12	1.67 ± 0.28	0.0162	90.7 ± 2.2
			P=0.041		

[a] Study was performed at SITEK Research Laboratories, Inc. The detailed protocol is presented by MacGregor *et al.* (1990).
[b] NCE=normochromatic erythrocyte
[c] Mean ± standard error
[d] Pairwise comparison with the controls, significant at P≤0.005 (ILS, 1990)
[e] Vehicle control
Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test, significant at P≤0.025 (ILS, 1990)

1.1.2.3 [Anonymous, 2018]

Study reference:

Anonymous (2018). TMPTA : mouse alkaline comet assay.

Detailed study summary and results:

Test type

Mouse alkaline Comet assay

GLP, similar to OECD 489

Test substance

- TMPTA
- Degree of purity = 80.2%
- Batch number F0994478VS

Test animals

- CD-1 female mice; Females only used in the Main Experiment (due to the appearance of hepatocholangio-carcinoma, stromal polyp and sarcoma observed in a previous carcinogenicity study in females only. There were no substantial gender differences in toxicity in the initial range-finder experiment.
- 6/dose; 3 for positive control group
- 25-32 g for the main experiment 1 and 26-36g for the main experiment 2.

Administration/exposure

- Vehicle: Polyethylene glycol 400 - The vehicle was selected as the test article was insoluble in water. As the dose route for this study was intravenous, formulations used on other toxicology studies with the same test article were considered incompatible. According to laboratory, they had previously dosed PEG400 intravenously in rats and they knew that it was a suitable vehicle for TMPTA. Prior to commencing any test article dosing, the vehicle PEG400 was initially attempted in a group of two male and two female mice in order to demonstrate tolerability intravenously.
- Slow intravenous; Two administrations at 0 (Day 1) and 23.5 (Day 2) hours.
- Experiment 1: 2.5, 5 and 10 mg/kg
- Experiment 2: 5, 10, 20 mg/kg

The following dose levels were tested in the Range-Finder Experiments:

Group No.	Group Description	Dose Volume (mL/kg)	Dose Level (mg/kg/day)	Animals ID	
				Male	Female
1RF	PEG400	2	0	M5001-M5002	M7001-M7002
2RF	TMPTA	2	20	M5101-M5103	M7101-M7103
3RF	TMPTA	2	30	M5201-M5203	-
3RF	TMPTA	2	10	-	M7201-M7203
4RF	TMPTA	2	10	-	M7301-M7303
5RF	TMPTA	2	20	-	M7401-M7403
6RF	TMPTA	2	30	-	M7501-M7503

The following dose levels were tested in Experiments 1 and 2:

Experiment	Group	Group Description	Dose Level (mg/kg/day)	Genotoxicity Animals	
				Animal ID Female	Sample Time
1	1	Vehicle control	0	M0001-M0006	Day 2 (24 hours)
	2	TMPTA	2.5	M0101-M0106	Day 2 (24 hours)
	3	TMPTA	5	M0201-M0206	Day 2 (24 hours)
	4	TMPTA	10	M0301-M0306	Day 2 (24 hours)
	5	Positive control	150	M0401-M0403	Day 2 (24 hours)
2	6	Vehicle control	0	M0501-M0506	Day 2 (24 hours)
	7	TMPTA	5	M0601-M0606	Day 2 (24 hours)
	8	TMPTA	10	M0701-M0706	Day 2 (24 hours)
	9	TMPTA	20	M0801-M0806	Day 2 (24 hours)
	10	Positive control	150	M0901-M0903	Day 2 (24 hours)

- Dose volume: 2 mL/kg for the vehicle control and test article treated Groups; 10 mL/kg for the positive control group.
- Sampling (liver and bone marrow) 30 minutes after last dose, on day 2.
- Positive (Ethyl methanesulfonate 150 mg/kg. Single oral administration at 21 hours (Day 2) and negative (vehicle/solvent) control data
- Scoring was carried out using fluorescence microscopy at an appropriate magnification and with suitable filters. A slide from a vehicle and positive control animal were checked for quality and/or response prior to analysis. All slides were allocated a random code and analysed by an individual not connected with the dosing phase of the study. All available animals per group were analysed. Measurements of tail intensity (%DNA in tail) were obtained from 150 cells/animal/tissue. In general this was evenly split over two or three slides.
- After completion of microscopic analysis and decoding of the data the:
    - Percentage tail intensity (i.e. %DNA in the tail) was calculated.

Data were treated as follows:

1. The median value per slide was calculated

2. The mean of the slide medians was calculated to give the mean animal value

3. The mean of the animal means and standard error of the mean was calculated for each group.

Tail intensity data for each slide were supplied for statistical analysis. The median of the log-transformed tail intensities from each slide was averaged to give an animal summary statistic. Where the median value on a slide was zero, a small constant (0.0001) was added before taking the logarithm and calculating the average for the animal. This animal average was used in the statistical analysis. Data was analysed using one-way analysis of variance (ANOVA) with the fixed factor for treatment group. The positive control group was excluded from this analysis. Levene's test was used to test for equality of variances among groups. This showed no evidence of heterogeneity (P>0.01). Comparisons between each treated roup and control were made using Dunnett's test. The test was one-sided looking for an increase in response with increasing dose. The back-transformed difference and p-value are reported. In addition, a linear contrast was used to test for an increasing dose response. The positive control groups were compared to the vehicle control groups using a two-sample t-test. Levene's test was used to test for equality of variances between the roups. This showed no evidence of heterogeneity (P>0.01). In both Experiments 1 and 2, the test was one-sided looking for an increase in response with increasing dose. The back-transformed difference and p-value are reported.

Results and discussion

- Maximum tolerated dose based on Range-Finder data
- Initial range-findgin experiments
    - First study: groups of two or three male and two or three female animals were dosed with vehicle or TMPTA at 10 (females only), 20 mg/kg/day (males and females) or 30 mg/kg/day (males only). Administration of the vehicle (PEG400) in two male and two female animals was well tolerated with no clinical signs of toxicity and no notable effects on bodyweight. At 20 mg/kg/day, following the first administration, clonic convulsions and gasping were noted in 1/3 males immediately post dose. However, the animal recovered by 0.5 hours post-dose. No clinical signs were observed in the females following the first dose. Following the second administration, no clinical signs were observed in the males. Clonic convulsions, gasping and shallow respiration were observed in 2/3 females immediately following the second dose and subsequently died. The remaining female survived to the end of the observation period. Of the surviving animals all lost some bodyweight (males lost between -3.2% and 5.8% and the female lost -6.0%). At 30 mg/kg/day in males, no clinical signs were observed following the first dose. Immediately following the second dose twitching was observed in 3/3 animals and pale extremities in 1/3 animals. Pale extremities was observed in 3/3 animals at 0.5 hours post-dose but all animals were back to a normal state by 1 hour post-dose. All animals lost bodyweight (between -1.6% to -4.1%). At 10 mg/kg/day in females, no clinical signs were observed following the first administration. Immediately following the second dose, 2/3 animals showed signs of rapid breathing. At 0.5 hours post-dose one of the animals was found missing its protective tail cuff and was sent to necropsy. Of the two remaining animals 1/2 animals had piloerection at 0.5 hours post-dose but was

back to a normal state by 1 hour post-dose. The other did not show any further clinical signs. All surviving animals lost bodyweight (between -1.1% to -8.1%). Clonic convulsions and twitching were considered to be dose limiting in the initial Range-Finder Experiments. As no significant sex differences were observed, Main Experiment 1 was conducted in female animals only.

  - Second study: At 10 mg/kg/day, there were no clinical signs of toxicity. All animals lost bodyweight (between -1.5% and -4.7%). At 20 mg/kg/day, there were no clinical signs of toxicity following the first dose. Following the second dose, hunched posture was observed in 1/3 animals at 2 to 4-6 hours. All animals lost bodyweight (between -5.0% and -7.7%). At 30 mg/kg/day, following the first dose 1/3 animals suffered a clonic convulsion immediately following the first dose. The animal was back to a normal state by 0.5 hours post-dose. Following the second dose, 3/3 animals suffered a clonic convulsion accompanied by gasping immediately following dosing. All animals were back to a normal state by 0.5 hours post-dose. At 4-6 hours post-dose, clinical signs of toxicity were observed in all animals including hunched posture (3/3), piloerection (1/3), lethargy (1/3), ataxia (1/3), laboured breathing (1/3) and hypothermia (1/3). All animals lost bodyweight (between 0.3% and 14.1%). Clinical signs including clonic convulsions and severe bodyweight loss were considered to be dose limiting in the repeated Range-Finder Experiments in females.

- Formulation analysis:
  - Experiment 1: Formulations generally fell outside the acceptance criteria for achieved concentration and homogeneity. No test article was detected in the vehicle control sample. Thus it was not possible to demonstrate exactly what the animals had been administered. Following these results, a second experiment was carried out in which the formulation were stirred for at least 30 minutes prior to and continuously (on a magnetic stirrer) during dosing.
  - Experiment 2: Achieved concentration and homogeneity of all formulations were within specification. No test article was detected in the vehicle control sample.
- Toxicity:
  - Experiment 1: none
  - Experiment 2: Clinical signs were generally limited to immediately following the first dose at all dose levels and included prostrate, rapid and/or gasping respiration, staggering, lethargic and dark eyes. Three animals (two control females and one female treated at 10 mg/kg/day) had a minor convulsion, but recovered and were kept on the study.
- No clinical chemistry findings were recorded in animals administered TMPTA. No macroscopical or microscopical changes related to administration of TMPTA.
- Other observations: Following dosing on Day 1, the pinport became detached from the catheter for two vehicle control animals (M0501 and M0503). At dosing on Day 2 these animals had fluid leaking from the protective tail cuff. Therefore, these animals only received a partial dose on Day 2. At dosing on Day 1, vehicle control animal M0504, it was seen that the catheter was blocked. At dosing on Day 2 this animal had fluid leaking from the protective tail cuff. Therefore, this animal only received a partial dose on Days 1 and 2. Following dosing on Days 1 and 2, low dose animal

M0603 had fluid leaking from the protective tail cuff during the saline flush. Therefore, this animal only received a partial dose on Days 1 and 2.

- No dose related increases in %hedgehogs in liver and bone marrow, thus demonstrating that treatment with TMPTA did not cause excessive DNA damage that could have interfered with comet analysis.
- Genotoxic effects
    - In Main Experiments 1 and 2, in the liver, female group mean tail intensity and tail moment values for all groups treated with TMPTA were comparable with the group mean vehicle control data. There were no statistically significant increases in tail intensity between treated and the concurrent vehicle control group.
    - In Experiment 1, in the bone marrow, female group mean tail intensity and tail moment values for all groups treated with TMPTA were comparable with the group mean vehicle control data. There were no statistically significant increases in tail intensity between treated and the concurrent vehicle control group.
    - In Experiment 2, in the bone marrow, the low and intermediate doses of 5 and 10 mg/kg/day did exhibit statistical significance (P≤0.01) although there was no evidence of a dose response.
    - Concurrent vehicle and positive control data valid (within historical control data)

Text Table 1: TMPTA: Summary of Group Mean Data – Liver, Experiment 1

Group / Dose Level (mg/kg/day)	Total No. Cells Scored	Tail Intensity Mean	SEM	Back-Transformed Difference from Vehicle	P-value	Significance	Mean %Hedgehogs
1F / Vehicle (0)	900	0.37	0.21	-	-		0.72
2F / TMPTA (2.5)	900	0.28	0.11	1.03	0.7286	NS	0.30
3F /TMPTA (5)	894	0.24	0.06	0.89	0.8244	NS	0.10
4F /TMPTA (10)	900	0.35	0.10	1.47	0.4341	NS	0.10
5F / EMS (150)	450	13.35	0.57	76.76	0.0003	P≤0.001	0.20

Dose Response (Groups 1, 2, 3 & 4): 0.2712 (NS)
F Female
SEM Standard error of means
NS P>0.05

Text Table 2: TMPTA: Summary of Group Mean Data – Bone Marrow, Experiment 1

Group / Dose Level (mg/kg/day)	Total No. Cells Scored	Tail Intensity Mean	SEM	Back-Transformed Difference from Vehicle	P-value	Significance	Mean %Hedgehogs
1F / Vehicle (0)	900	0.29	0.09	-	-		0.08
2F / TMPTA (2.5)	900	0.16	0.03	0.90	0.8456	NS	0.08
3F /TMPTA (5)	900	0.22	0.06	0.89	0.8511	NS	0.24
4F /TMPTA (10)	900	0.16	0.02	0.86	0.8727	NS	0.23
5F / EMS (150)	450	10.74	1.74	71.66	<0.0001	P≤0.001	0.45

Dose Response (Groups 1, 2, 3 & 4): 0.6526 (NS)
F Female
SEM Standard error of means
NS P>0.05

Text Table 3: TMPTA: Summary of Group Mean Data – Liver, Experiment 2

Group / Dose Level (mg/kg/day)	Total No. Cells Scored	Tail Intensity Mean	SEM	Back-Transformed Difference from Vehicle	P-value	Significance	Mean %Hedgehogs
6F / Vehicle (0)	900	0.48	0.08	-	-	-	0.52
7F / TMPTA (5)	900	0.31	0.12	0.74	0.9438	NS	0.21
8F /TMPTA (10)	900	0.24	0.05	0.58	0.9886	NS	0.54
9F /TMPTA (20)	900	0.59	0.17	1.44	0.3384	NS	0.21
10F / EMS (150)	450	17.60	1.78	62.34	<0.0001	P≤0.001	3.16

Dose Response (Groups 6, 7, 8 & 9): 0.2396 (NS)
F Female
SEM Standard error of means
NS P>0.05

Text Table 4: TMPTA: Summary of Group Mean Data – Bone Marrow, Experiment 2

Group / Dose Level (mg/kg/day)	Total No. Cells Scored	Tail Intensity Mean	SEM	Back-Transformed Difference from Vehicle	P-value	Significance	Mean %Hedgehogs
6F / Vehicle (0)	900	0.18	0.03	-	-	-	0.39
7F / TMPTA (5)	900	0.67	0.23	2.95	0.0001	P≤0.001	0.35
8F /TMPTA (10)	900	0.29	0.03	2.05	0.0060	P≤0.01	0.07
9F /TMPTA (20)	900	0.25	0.05	1.11	0.5668	NS	0.13
10F / EMS (150)	450	9.43	0.23	78.57	<0.0001	P≤0.001	0.99

Dose Response (Groups 6, 7, 8 & 9): 0.5365 (NS)
F Female
SEM Standard error of means
NS P>0.05

9.3 Historical Control Ranges: Comet Assay Data

Data generated from studies performed within the GLP laboratory, by GLP trained staff, whether a claim of GLP compliance was made or not, were included in the compilation of the historical control ranges without bias.

MOUSE LIVER COMET HISTORICAL CONTROL RANGES			
Vehicle Control Data			
		Tail Intensity (%)	**Hedgehogs (%)**
Number of Animals		78	72
Mean		1.28	4.07
Standard Deviation		1.37	3.37
Observed Range	**Minimum**	0.04	0.00
	Maximum	6.91	14.53
95% Reference Range	**Lower Limit**	0.05	0.00
	Upper Limit	5.30	11.30
Positive Control Data			
		Tail Intensity (%)	**Hedgehogs (%)**
Number of Animals		64	59
Mean		21.89	9.55
Standard Deviation		18.06	7.00
Observed Range	**Minimum**	2.99	0.00
	Maximum	66.61	22.28
95% Reference Range	**Lower Limit**	4.50	0.73
	Upper Limit	63.81	21.32

Range compiled December 2016; generated from 13 studies dosed between January 2008 and June 2015

MOUSE BONE MARROW COMET HISTORICAL CONTROL RANGES			
Vehicle Control Data			
		Tail Intensity (%)	**Hedgehogs (%)**
Number of Animals		5	5
Mean		0.44	7.38
Standard Deviation		0.18	2.30
Observed Range	**Minimum**	0.24	3.37
	Maximum	0.72	8.88
95% Reference Range	**Lower Limit**	NA	NA
	Upper Limit	NA	NA
Positive Control Data			
		Tail Intensity (%)	**Hedgehogs (%)**
Number of Animals		3	3
Mean		11.45	10.06
Standard Deviation		2.44	0.69
Observed Range	**Minimum**	8.68	9.55
	Maximum	13.27	10.84
95% Reference Range	**Lower Limit**	NA	NA
	Upper Limit	NA	NA

Range compiled December 2016; generated from 1 study dosed in June 2015

- To be noted that historical vehicle control data for bone marrow consist of male CD-mice dosed orally with carboxymethyl cellulose, and not PEG 400.

1.2 Carcinogenicity

1.2.1 Animal data

1.2.1.1 [Anonymous, 1982]

Study reference:

Anonymous (1982). Chronic mouse dermal toxicity study.

Detailed study summary and results:

Test type

Non-guideline study; GLP not specified

Test substance

- TMPTA (C-92)
- Degree of purity: not stated

Test animals

- C3H/HeJ male mice
- 50/group
- Age at the study initiation: 6-8 weeks

Administration/exposure

- A group of 50 C3H/HeJ male mice received topical application of 50 mg of C-92 (5 % in white mineral oil) to shaved area of the back, twice weekly for 80 weeks.
- A group of solvent control and positive control (0.05% benzo(a)pyrene in mineral oil) were also maintained along with no-treatment control group in the study.
- Parameters evaluated included clinical signs, mortality, body weight, gross pathology and histopathological (neoplastic and non-neoplastic) examinations.

Results and discussion

- CLINICAL SIGNS AND MORTALITY: Nearly all the mice survived the first three months of exposures. Percent survival after 78 week was 60.
- GROSS PATHOLOGY: Dark red lesions in lungs, liver tumors, kidney haemorrhages, enlarged spleen, skin ulcers, flaky skin, enlarged and gray lymph nodes, haemorrhages in stomach, gray or yellow spots in adrenals
- HISTOPATHOLOGY: NON-NEOPLASTIC: Lesions include ulcer, abscess, acanthosis, dysplasia, fibrosis, pigmentation, hyperkeratosis, retention cysts
- HISTOPATHOLOGY: NEOPLASTIC:
    - No-treatment group: Liver carcinoma (18 mice), lymphadenitis (0 mice)
    - Solvent control group: Liver carcinoma (13 mice), lymphadenitis (2 mice)
    - Treatment group (C-92): Liver carcinoma (2 mice), lymphadenitis (2 mice) but no skin tumors

1.2.1.2 [NTP, 2005]

Study reference:

NTP (2005). NTP report on the toxicology studies of trimethylolpropane triacrylate (technical grade) (CAS No. 15625-89-5) in F344/N rats, B6C3F1 mice and genetically modified (FVB Tg. Ac hemizygous) mice (dermal studies).

Detailed study summary and results:

Test type

Not guideline; GLP compliant

Test substance

- TMPTA
- Degree of purity = 80%
- GC indicated one major peak and two impurities with areas of 6.5% and 3.4% relative to the major peak area. HPLC indicated a major peak and five impurities with a combined area of 22.2%. HPLC/MS indicated 10 impurities contributing or corresponding to the impurity peaks identified by HPLC. These impurities included four structurally related acrylates or adducts: trimethylolpropane diacrylate, trimethylolpropane triacrylate acrylic acid adduct, trimethylolpropane triacrylate-trimethylolpropane monoacrylate adduct, and trimethylolpropane triacrylatetrimethylolpropane diacrylate adduct. No substantial amount of 4-methoxyphenol, a stabilizer added to trimethylolpropane triacrylate, was detected.
- Batch number: 01031AW

Test animals

- mouse (Tg.AC hemizygous) male/female
- 15/sex/group

Administration/exposure

- Application on the backs of male and female Tg.AC mice five times per week for 6 months.
- 0, 0.75, 1.5, 3, 6, 12 mg/kg (nominal conc.)
- Animals painted with acetone alone served as the control groups.
- Tissues from 15 sites were examined for every animal.

TABLE 1
Experimental Design and Materials and Methods in the Dermal Studies of Trimethylolpropane Triacrylate

2-Week Studies	3-Month Studies	6-Month Study
Study Laboratory Battelle Columbus Laboratories (Columbus, OH)	Battelle Columbus Laboratories (Columbus, OH)	Battelle Columbus Laboratories (Columbus, OH)
Strain and Species F344/N rats $B6C3F_1$ mice	F344/N rats $B6C3F_1$ mice	Tg.AC [FVB/N-TgN(V-Ha-*ras*)] hemizygous mice
Animal Source Taconic Laboratory Animals and Services (Germantown, NY)	Taconic Laboratory Animals and Services (Germantown, NY)	Taconic Laboratory Animals and Services (Germantown, NY)
Time Held Before Studies Rats: 11 days Mice: 12 days	Rats: 11 days (males) or 12 days (females) Mice: 13 days (females) or 14 days (males)	11 days
Average Age When Studies Began 6 weeks	6 weeks	6 weeks
Date of First Dose Rats: May 6, 1996 Mice: May 7, 1996	Rats: September 9 (male) or 10 (female), 1996 Mice: September 11 (female) or 12 (male), 1996	July 20, 1998
Duration of Dosing 5 days per week for 16 days	5 days per week for 14 weeks	Core study: 5 days per week for 28 weeks Positive control: 3 days per week for 28 weeks
Date of Last Dose Rats: May 21, 1996 Mice: May 22, 1996	Rats: December 10 (male) or 11 (female), 1996 Mice: December 12 (female) or 13 (male), 1996	Core study: January 27 (male) or 28 (female), 1999 Positive control: January 28, 1999
Necropsy Dates Rats: May 22, 1996 Mice: May 23, 1996	Rats: December 10 (male) or 11 (female), 1996 Mice: December 12 (female) or 13 (male), 1996	Core study: January 27 (male) or 28 (female), 1999 Positive control: January 28, 1999
Average Age at Necropsy 8 weeks	Rats: 19 weeks Mice: 19 (female) or 20 (male) weeks	33 weeks
Size of Study Groups 5 males and 5 females	10 males and 10 females	15 males and 15 females
Method of Distribution Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 2-week studies	Same as 2-week studies
Animals per Cage 1	1	1

TABLE 1
Experimental Design and Materials and Methods in the Dermal Studies of Trimethylolpropane Triacrylate

2-Week Studies	3-Month Studies	6-Month Study
Method of Animal Identification Tail tattoo	Tail tattoo	Tail tattoo
Diet NTP-2000 pelleted diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, changed weekly	Same as 2-week studies, except feed was irradiated	Same as 3-month studies
Water Tap water (Columbus municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available *ad libitum*	Same as 2-week studies	Same as 2-week studies
Cages Polycarbonate (Lab Products, Inc., Maywood, NJ), changed at least once per week, rotated every 2 weeks	Same as 2-week studies	Same as 2-week studies
Bedding Sani-Chip® hardwood chips (P.J. Murphy Forest Products Corp., Montville, NJ), changed at least once per week	Same as 2-week studies, except bedding was irradiated	Same as 3-month studies
Cage Filters Spun-bonded DuPont 2024 polyester (Snow Filtration Co., Cincinnati, OH), changed every 2 weeks	Same as 2-week studies	Same as 2-week studies
Racks Stainless steel, cleaned and rotated every 2 weeks	Same as 2-week studies	Same as 2-week studies
Animal Room Environment Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour
Doses 0, 12.5, 25, 50, 100, or 200 mg/kg in acetone by dermal application (dosing volume 0.5 mL/kg for rats and 2 mL/kg for mice)	0, 0.75, 1.5, 3, 6, or 12 mg/kg in acetone by dermal application (dosing volume 0.5 mL/kg for rats and 2 mL/kg for mice)	Core study: 0, 0.75, 1.5, 3, 6, or 12 mg/kg in acetone by dermal application (dosing volume 3.3 mL/kg) Positive control: 1.25 µg 12-*O*-tetradecanoylphorbol-13-acetate/100 mL acetone by dermal application (dosing volume 100 µL)
Type and Frequency of Observation Animals were observed twice daily and were weighed initially, on day 8, and at the end of the studies. Clinical findings were recorded daily.	Animals were observed twice daily and were weighed initially, weekly, and at the end of the studies. Clinical findings were recorded weekly for core study animals.	Animals were observed twice daily and were weighed initially, weekly, and at the end of the study. Clinical findings were recorded weekly and at the end of the study. Observations of papilloma formation on the skin were recorded weekly.

TABLE 1
Experimental Design and Materials and Methods in the Dermal Studies of Trimethylolpropane Triacrylate

2-Week Studies	3-Month Studies	6-Month Study
Method of Sacrifice Carbon dioxide asphyxiation	Same as 2-week studies	Same as 2-week studies
Necropsy Necropsies were performed on all animals. Organs weighed were the heart, right kidney, liver, lung, right testis, and thymus.	Necropsies were performed on all animals. Organs weighed were the heart, right kidney, liver, lung, right testis, and thymus.	Necropsies were performed on core study mice. Organs weighed were the heart, right kidney, liver, lung, right testis, and thymus.
Clinical Pathology None	Blood was collected from the retroorbital sinus of special study rats on days 4 and 23 and from core study rats and mice at the end of the studies for hematology and clinical chemistry (rats only). ***Hematology:*** hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, and platelet counts; erythrocyte morphology; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials ***Clinical chemistry:*** urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and bile acids	None
Histopathology Histopathologic examinations were performed on the skin (site of application) of all animals and the thymus of all mice.	Complete histopathologic examinations were performed on vehicle control and 12 mg/kg rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, gallbladder (mice), heart with aorta, large intestine (cecum, colon, and rectum), small intestine (duodenum, jejunum, and ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin (site of application), spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus. The skin at the site of application was also examined in the remaining core study groups.	Histopathologic examinations were performed on all core study mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, heart, kidney, liver, lung, lymph nodes (mandibular, mediastinal, and mesenteric), ovary, pituitary gland, skin (site of application and inguinal), spleen, stomach (forestomach), testis with epididymis, thymus, thyroid gland, and uterus.

Results and discussion

Summary of the 6-Month Toxicology and Genetic Toxicology Studies of Trimethylolpropane Triacrylate

	Male Tg.AC Hemizygous Mice	Female Tg.AC Hemizygous Mice
Doses in acetone by dermal application	Vehicle control, 0.75, 1.5, 3, 6, or 12 mg/kg	Vehicle control, 0.75, 1.5, 3, 6, or 12 mg/kg
Body weights	Dosed groups similar to the vehicle control group	Dosed groups similar to the vehicle control group
Survival rates	14/15, 15/15, 12/15, 14/15, 13/15, 11/15	15/15, 14/15, 12/15, 14/15, 14/15, 12/15
Nonneoplastic effects	Skin (site of application): epidermal hyperplasia (0/15, 0/15, 0/15, 6/15, 14/15, 15/15); hyperkeratosis (0/15, 0/15, 1/15, 15/15, 14/15, 12/15); chronic active inflammation (0/15, 0/15, 1/15, 1/15, 9/15, 12/15) All organs: myelodysplasia (0/15, 0/15, 0/15, 0/15, 0/15, 2/15)	Skin (site of application): epidermal hyperplasia (0/15, 0/15, 1/15, 4/15, 15/15, 15/15); hyperkeratosis (0/15, 0/15, 1/15, 7/15, 14/15, 13/15); chronic active inflammation (0/15, 0/15, 0/15, 3/15, 14/15, 12/15) All organs: myelodysplasia (0/15, 0/15, 0/15, 0/15, 0/15, 2/15)
Neoplastic effects	Skin (site of application): squamous cell papilloma (0/15, 0/15, 0/15, 2/15, 12/15, 13/15)	Skin (site of application): squamous cell papilloma (0/15, 0/15, 0/15, 1/15, 11/15, 15/15); squamous cell carcinoma (0/15, 0/15, 1/15, 0/15, 1/15, 1/15)
Uncertain findings	None	Forestomach: squamous cell papilloma (4/15, 5/15, 4/15, 2/15, 5/15, 9/15)
Genetic toxicology		
Micronucleated erythrocytes		
Mouse peripheral blood *in vivo:*		
$B6C3F_1$	Negative in males and females	
Tg.AC hemizygous	Negative in males and females	

- Survival: no effect

TABLE 10
Survival of Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Male						
Animals initially in study	15	15	15	15	15	15
Natural deaths	1	0	3	1	2	4
Animals surviving to study termination	14	15	12	14	13	11
Percent probability of survival at end of study[a]	93	100	80	93	87	73
Mean survival (days)[b]	186	192	175	188	185	187
Survival analysis[c]	P=0.111	P=1.000N	P=0.567	P=1.000N	P=0.984	P=0.357
Female						
Animals initially in study	15	15	15	15	15	15
Moribund	0	0	1	0	0	0
Natural deaths	0	1	2	1	1	3
Animals surviving to study termination	15	14	12	14	14	12
Percent probability of survival at end of study	100	93	80	93	93	80
Mean survival (days)	193	192	171	188	191	187
Survival analysis[c]	P=0.305	P=1.000	P=0.226	P=1.000	P=1.000	P=0.224

[a] Kaplan-Meier determinations
[b] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[c] The result of the life table trend test (Tarone, 1975) is in the vehicle control column, and the results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the dosed group columns. A lower mortality in a dosed group is indicated by **N**.

- Body weight and clinical signs: Mean body weights of dosed groups of mice were 95% to 105% those of the vehicle controls for most of the study. Treatment-related clinical findings included papillomas at the site of application in 3 mg/kg and greater males and 6 and 12 mg/kg females; one female administered 1.5 mg/kg also had a papilloma. The number of mice with papillomas, the total number of papillomas, and the mean number of papillomas per mouse increased with

increasing dose. Papillomas were observed earlier in the 6 and 12 mg/kg groups than in the other dose groups.

Mean Body Weights and Survival of Male Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control		0.75 mg/kg			1.5 mg/kg			3 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
Mean for weeks											
1-13	26.8		25.9	97		27.0	101		26.7	100	
14-28	31.5		30.8	98		32.9	104		32.7	104	

Weeks on Study	6 mg/kg			12 mg/kg		
	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
Mean for weeks						
1-13	26.3	98		26.4	99	
14-28	31.0	98		31.7	100	

Mean Body Weights and Survival of Female Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate

Weeks on Study	Vehicle Control		0.75 mg/kg			1.5 mg/kg			3 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
Mean for weeks											
1-13	22.7		22.8	101		23.0	101		22.8	100	
14-28	26.6		26.6	100		27.3	103		26.9	101	

Weeks on Study	6 mg/kg			12 mg/kg		
	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
Mean for weeks						
1-13	22.7	100		22.5	99	
14-28	26.7	100		27.0	102	

- Organ weights and organ weight to body weight ratio: Liver weights were increased in 12 mg/kg male and female mice. Absolute and relative lung weights of 6 and 12 mg/kg males and 12 mg/kg females were significantly less than those of the vehicle controls; absolute lung weights were also decreased in 6 mg/kg females. Heart weights of 12 mg/kg males and females (absolute only) were significantly increased. Females administered 12 mg/kg also had significantly increased kidney weights.

Skin Papilloma Formation at the Site of Application in Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate[a]

Dose (mg/kg)	Number (Percent) with Papilloma[b]		Time to Initial Papilloma Occurrence for All Animals in Group[c] (Week)		Distribution of Number of Papillomas per Animal[d] Quantiles			Test of Dunson *et al.* Model[e]	
			First	Median	20th	50th	80th	γ_1	γ_2
Male									
0	0	(0.0%)	NA	>28	0	0	0		
0.75	0	(0.0%)	NA	>28	0	0	0		NT
1.5	0	(0.0%)	NA	>28	0	0	0		NT
3	3	(20.9%)	23	>28	0	0	0		NT
6	13	(91.9%)	14	20	1	6	15.5	**	NT
12	13	(86.7%)	9	13	20+	20+	20+	**	NT
Trend								**	**
Positive Control[f]	15	(100.0%)	9	10	9	20+	20+	**	NT
Female									
0	0	(0.0%)	NA	>28	0	0	0		
0.75	0	(0.0%)	NA	>28	0	0	0		NT
1.5	1	(8.1%)	19	>28	0	0	0		NT
3	2	(14.0%)	28	>28	0	0	0		NT
6	12	(80.0%)	9	25	0	1	4	**	NT
12	15	(100.0%)	10	14	20+	20+	20+	**	NT
Trend								**	**
Positive Control	14	(99.8%)	9	10	1	16	20+	**	NT

[a] 15 males and 15 females initially in each dose group
[b] Percent is Poly-3 adjusted rate and reflects whether the animal ever had a confirmed papilloma at any point in the study.
[c] For groups in which fewer than half of the animals had papillomas, the median time to initial occurrence is >28 weeks. NA=not applicable.
[d] Quantiles are based on all animals in a group, whether removed before the end of study or not. For example, a value of 9 for the 20th quantile implies that 20% of the animals in the study had 9 papillomas or fewer at the end of the study (or at removal from study).
[e] The Dunson *et al.* (2000) model accounts for latency (γ_1) and multiplicity (γ_2) in the rate of occurrence, NT (No Test) indicates that a pairwise test could not be meaningfully applied because no papillomas were observed in the control group. ** ($P \leq 0.01$) indicates a significant trend or a significant difference from the vehicle control group.
[f] 100 µL of 1.25 µg 12-*O*-tetradecanoylphorbol-13-acetate per 100 mL acetone administered three times per week.

- Pathology and statistical analysis
- *Skin*: Squamous cell neoplasms at the site of application were associated with dermal application of trimethylolpropane triacrylate. The incidences of squamous cell papilloma were significantly increased in 6 and 12 mg/kg males and females. Papillomas were multiple in all 12 mg/kg males and females and 6 mg/kg males. Two males and one female in the 3 mg/kg groups also had squamous cell papillomas at the site of application. Squamous cell papilloma had the typical morphology of an exophytic growth of well-differentiated squamous epithelium covering arborizing fronds of connective tissue. Multiple papillomas were often contiguous. One female in each of the 1.5, 6, and 12 mg/kg groups also had a squamous cell carcinoma at the site of application. These carcinomas appeared to arise within papillomas, with extensions of atypical squamous cells into the underlying dermis and subcutis. Squamous cell carcinomas at the site of application were considered to be treatment-related and possibly the result of malignant conversion of papillomas.
- Nonneoplastic effects at the site of application were squamous cell hyperplasia, hyperkeratosis, and chronic active inflammation. Hyperplasia was present in the 3 mg/kg and greater groups and was characterized by increased thickness of the epidermis, from the normal one to three cell layers to four to six cell layers thick (minimal to mild severity). The hyperplasia was generally diffuse in areas between papillomas. Occasionally a focal nodular type of hyperplasia was

observed that was considered a precursor lesion to squamous papilloma. Hyperplasia was accompanied by minimal to mild increased thickness of the keratin layer (hyperkeratosis). A minimal to mild infiltrate of mixed inflammatory cells (chronic active inflammation) was present in the dermis, generally in the 6 and 12 mg/kg groups. Some inflammation was invariably associated with neoplasms, but the diagnosis was made when infiltrates were observed in nonneoplastic areas. Sebaceous glands at the site of application were enlarged (hyperplasia) in several males and females administered 3 mg/kg or greater, but this lesion was not diagnosed separately.

Incidences of Neoplasms and Nonneoplastic Lesions of the Skin (Site of Application) in Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Male						
Number Examined						
Microscopically	15	15	15	15	15	15
Epidermis, Hyperplasia[a]	0	0	0	6** (1.0)[b]	14** (1.7)	15** (1.9)
Hyperkeratosis	0	0	1 (2.0)	15** (1.0)	14** (1.1)	12** (1.7)
Inflammation, Chronic Active	0	0	1 (1.0)	1 (1.0)	9** (1.1)	12** (1.3)
Squamous Cell Papilloma, Multiple	0	0	0	0	12**	13**
Squamous Cell Papilloma (includes multiple)						
Overall rate[c]	0/15 (0%)	0/15 (0%)	0/15 (0%)	2/15 (13%)	12/15 (80%)	13/15 (87%)
Adjusted rate[d]	0.0%	0.0%	0.0%	14.0%	85.1%	86.7%
Terminal rate[e]	0/14 (0%)	0/15 (0%)	0/12 (0%)	2/14 (14%)	11/13 (85%)	9/11 (82%)
First incidence (days)	—[g]	—[h]	—	192 (T)	180	161
Poly-3 test[f]	P<0.001	—	—	P=0.234	P<0.001	P<0.001
Female						
Number Examined						
Microscopically	15	15	15	15	15	15
Epidermis, Hyperplasia	0	0	1 (2.0)	4* (1.0)	15** (1.2)	15** (2.0)
Hyperkeratosis	0	0	1 (1.0)	7** (1.0)	14** (1.1)	13** (1.6)
Inflammation, Chronic Active	0	0	0	3 (1.0)	14** (1.0)	12** (1.3)
Squamous Cell Papilloma, Multiple	0	0	0	0	5*	15**
Squamous Cell Papilloma (includes multiple)						
Overall rate	0/15 (0%)	0/15 (0%)	0/15 (0%)	1/15 (7%)	11/15 (73%)	15/15 (100%)
Adjusted rate	0.0%	0.0%	0.0%	7.0%	73.3%	100%
Terminal rate	0/15 (0%)	0/14 (0%)	0/12 (0%)	1/14	10/14 (71%)	12/12 (100%)
First incidence (days)	—	—	—	193 (T)	162	118
Poly-3 test	P<0.001	—	—	P=0.490	P<0.001	P<0.001
Squamous Cell Carcinoma	0	0	1	0	1	1

(T)Terminal sacrifice
* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Number of neoplasm-bearing animals/number of animals with skin examined microscopically
[d] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[e] Observed incidence at terminal kill
[f] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence is the P value corresponding to pairwise comparison between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice.
[g] Not applicable; no neoplasms in animal group
[h] Value of statistic cannot be computed.

- *Forestomach*: The incidence of squamous cell papilloma in 12 mg/kg females was significantly greater than that in the vehicle control group. The morphology of these neoplasms was similar to that of skin papillomas. Multiple papillomas occurred in three of the nine affected females in the 12

mg/kg group, in contrast to single incidences in all other groups. No other proliferative squamous lesions (hyperplasia or carcinoma) were observed. Forestomach papilloma is a relatively common spontaneous finding in Tg.AC mice that may occur at a high and variable rate (10%-25% in hemizygous females; higher in homozygous mice; Mahler et al., 1998). The increased incidence of forestomach papilloma in 12 mg/kg females may have been treatment related.

Incidences of Squamous Cell Papilloma of the Forestomach in Female Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Number Examined Microscopically	15	15	15	15	15	15
Squamous Cell Papilloma, Multiple[a]	1	1	1	1	1	3
Squamous Cell Papilloma (includes multiple)						
Overall rate[b]	4/15 (27%)	5/15 (33%)	4/15 (27%)	2/15 (13%)	5/15 (33%)	9/15 (60%)
Adjusted rate[c]	26.7%	33.7%	32.5%	14.0%	34.3%	64.6%
Terminal rate[d]	4/15 (27%)	5/14 (36%)	4/12 (33%)	2/14 (14%)	5/14 (36%)	9/12 (75%)
First incidence (days)	193 (T)	193 (T)	193 (T)	193 (T)	193 (T)	193 (T)
Poly-3 test[e]	P=0.014	P=0.493	P=0.535	P=0.352N	P=0.481	P=0.040

(T) Terminal sacrifice
[a] Number of animals with lesion
[b] Number of neoplasm-bearing animals/number of animals with forestomach examined microscopically
[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence is the P value corresponding to pairwise comparison between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A lower incidence in a dosed group is indicated by N.

- *Other Organs*: Incidences of hematopoietic cell proliferation in various tissues were increased in dosed mice. Hematopoietic cell proliferation was significantly increased in the liver of 12 mg/kg males and females and the spleen of 6 and 12 mg/kg males, as well as in the mandibular, mediastinal, and mesenteric lymph node of 12 mg/kg females. Hematopoietic cell proliferation consisted of increased numbers of erythroid and granulocytic precursors in the splenic red pulp, liver sinusoids, or nodal parenchyma. These changes were attributed to the dermal inflammatory stimulus at the application site and/or release of hematopoietic cytokines and growth factors from proliferative epidermal cells.

- There was a change that occurred in some animals administered 6 or 12 mg/kg. This change was observed in one or more organs and was characterized by somewhat variable morphology and uncertain biological behavior. Florid lesions, diagnosed as myelodysplasia, were identified in two males and two females exposed to 12 mg/kg, while milder lesions, diagnosed as cell, infiltration, nonspecified site, were identified in several animals. The change was characterized predominantly by myeloid infiltration/proliferation that tended to be perivascular in the liver and lungs. Infiltrating cells were predominantly mature and immature granulocytes (eosinophils and neutrophils) with lesser numbers of admixed mononuclear cells. In severely affected livers, there was bridging between portal tracts and accumulations of brightly eosinophilic crystalline material in the lumen of bile ductules. The change was commonly observed in the mediastinal, mandibular, axillary, and mesenteric lymph nodes, and often included a pronounced plasma cell population in the medullary sinuses. The epididymis and spleen were also often involved.

Incidences of Selected Nonneoplastic Lesions in Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control		0.75 mg/kg		1.5 mg/kg		3 mg/kg		6 mg/kg		12 mg/kg	
Male												
Liver[a]	15		15		15		15		15		15	
Hematopoietic Cell Proliferation[b]	1	(1.0)[c]	0		1	(1.0)	0		0		6*	(1.3)
Spleen	15		15		15		15		15		15	
Hematopoietic Cell Proliferation	1	(2.0)	4	(1.5)	1	(3.0)	2	(1.5)	6*	(2.0)	8**	(2.6)
All Organs[d]	15		15		15		15		15		15	
Myelodysplasia	0		0		0		0		0		2	
Infiltration Cellular	0		0		0		0		0		5*	
Infiltration Cellular, Plasma Cell	0		0		0		0		0		4*	
Female												
Liver	15		15		15		15		15		15	
Hematopoietic Cell Proliferation	0		0		1	(2.0)	0		0		6**	(1.8)
Lymph Node, Mandibular	15		15		15		15		15		15	
Hematopoietic Cell Proliferation	0		0		1	(3.0)	0		0		6**	(2.0)
Lymph Node, Mediastinal	11		14		13		10		13		12	
Hematopoietic Cell Proliferation	0		0		0		0		0		5*	(2.0)
Lymph Node, Mesenteric	15		14		14		15		15		15	
Hematopoietic Cell Proliferation	0		0		0		0		0		4*	(2.0)
All Organs	15		15		15		15		15		15	
Myelodysplasia	0		0		0		0		0		2	
Infiltration Cellular	0		0		1		0		0		3	
Infiltration Cellular, Plasma Cell	0		0		1		0		0		2	

* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[d] Number of animals with any tissue examined microscopically

1.2.1.3 [NTP, 2012]

Study reference:

NTP (2012). Toxicology and carcinogenesis studies of trimethylolpropane triacrylate (technical grade) in F344/N rats and B6C3F1/N mice.

Detailed study summary and results:

Test type

equivalent or similar to OECD Guideline 451

Test substance

- TMPTA
- Degree of purity > 78%
- *Impurities (or a note that the impurities do not affect the classification)*
- *Batch number*

Test animals

- F344/N rats and B6C3F1 mice male/female
- 65/sex/group

Administration/exposure

- 0.3, 1.0, 3.0mg/kg (nominal conc.)
- Vehicle: acetone
- Exposure: 104 to 105 weeks (rats); 105 to 106 weeks (mice)
- Dermal application (5 times per week)
- Interim evaluations performed after 2, 13 and 52 weeks

TABLE 4
Experimental Design and Materials and Methods in the 2-Year Dermal Studies of Trimethylolpropane Triacrylate

Study Laboratory
Southern Research Institute (Birmingham, AL)

Strain and Species
F344/N rats
B6C3F1/N mice

Animal Source
Taconic Farms, Inc. (Germantown, NY)

Time Held Before Studies
Rats: 13 days
Mice: 12 days

Average Age When Studies Began
Rats: 6 weeks
Mice: 5 to 6 weeks

Date of First Dose
Rats: January 18, 2005
Mice: December 13, 2004

Duration of Dosing
Rats: 5 days per week for 2 weeks, 13 weeks, or 12 months (interim evaluations) or 104 to 105 weeks
Mice: 5 days per week for 2 weeks, 13 weeks, or 12 months (interim evaluations) or 105 to 106 weeks

Date of Last Dose
Rats: January 15 to 21, 2007
Mice: December 10 to 18, 2006

Necropsy Dates
Rats: January 16 to 22, 2007
Mice: December 11 to 19, 2006

Average Age at Necropsy
Rats: 110-111 weeks
Mice: 109-111 weeks

Size of Study Groups
65 males and 65 females

Method of Distribution
Animals were distributed randomly into groups of approximately equal initial mean body weights.

Animals per Cage
1

Method of Animal Identification
Tail tattoo

Diet
Irradiated NTP-2000 open formula wafers (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*

Water
Tap water (Birmingham, AL, municipal supply) via automatic watering system (Edstrom Industries, Inc., Waterford, WI), available *ad libitum*

Cages
Solid-bottom polycarbonate (Lab Products, Inc., Maywood, NJ), changed weekly

Bedding
Heat-treated, irradiated hardwood chips (P.J. Murphy Forest Products, Corp., Montville, NJ), changed once weekly

Cage Filters
Reemay® spun-bonded polyester (Andico, Birmingham, AL), changed weekly

Racks
Stainless steel (Lab Products, Inc., Maywood, NJ), cleaned weekly and rotated every 2 weeks

Animal Room Environment
Temperature: 72° ± 3° F
Relative humidity: 50% ± 15%
Room fluorescent light: 12 hours/day
Room air changes: minimum 10/hour

Doses
0, 0.3, 1.0, or 3.0 mg/kg in acetone (dosing volume 0.5 mL/kg for rats and 2.0 mL/kg for mice)

Type and Frequency of Observation
Observed twice daily. Animals were weighed initially, approximately weekly for 13 weeks, monthly thereafter, and at the end of the studies. Clinical findings were recorded on day 3 (male rats), day 4 (female rats and male mice), day 5 (female mice), and at 4-week intervals beginning week 5.

Method of Kill
Carbon dioxide asphyxiation

Necropsy
Necropsies were performed on all core study animals.

Histopathology
Complete histopathology was performed on all core study rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone, brain, clitoral gland, esophagus, eyes, gallbladder (mice), Harderian gland, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung and mainstem bronchi, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin (site of application and control site), spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus. At 2 weeks, 13 weeks, and 12 months, groups of five male and five female rats and mice per dose group were evaluated for histological changes of the skin from the site of application.

- The concurrent control group represents the most valid comparison to the treated groups and is the only control group analyzed statistically in NTP bioassays. However, historical control data are often helpful in interpreting potential treatment-related effects, particularly for uncommon or rare neoplasm types. For meaningful comparisons, the conditions for studies in the historical database must be generally similar. One significant factor affecting the background incidence of neoplasms at a variety of sites is diet. In 1995, the NTP incorporated a new diet (NTP-2000) that contains less protein and more fiber and fat than the NIH-07 diet previously used in toxicity and carcinogenicity studies (Rao, 1996, 1997). The NTP historical database contains all studies that use the NTP-2000 diet with histopathology findings completed within the most recent 5-year period. A second potential source of variability is route of administration. In general, the historical database for a given study will include studies using the same route of administration, and the overall incidences of neoplasms for all routes of administration are included for comparison. For the current study, the incidences for dermal studies with all vehicles were combined because the historical database does not include any other dermal studies with acetone as the vehicle; these incidences and the overall incidences for all routes of administration were used for comparison.

Results and discussion

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Trimethylolpropane Triacrylate

	Male F344/N Rats	Female F344/N Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Concentrations in acetone by dermal application	0, 0.3, 1.0, or 3.0 mg/kg	0, 0.3, 1.0, or 3.0 mg/kg	0, 0.3, 1.0, or 3.0 mg/kg	0, 0.3, 1.0, or 3.0 mg/kg
Body weights	Dosed groups within 10% of the vehicle control group	Dosed groups within 10% of the vehicle control group	Dosed groups within 10% of the vehicle control group	Dosed groups within 10% of the vehicle control group
Survival rates	23/50, 18/50, 28/50, 23/50	27/50, 31/50, 24/50, 32/50	30/50, 35/50, 29/50, 38/50	39/50, 31/50, 30/50, 30/50
Nonneoplastic effects	Skin (site of application): epidermis, hyperplasia (1/50, 0/49, 12/50, 28/50); hyperkeratosis (2/50, 4/49, 33/50, 49/50)	Skin (site of application): epidermis, hyperplasia (0/50, 4/50, 11/50, 25/50); hyperkeratosis (0/50, 11/50, 42/50, 50/50)	Skin (site of application): epidermis, hyperplasia (10/50, 7/50, 15/50, 44/50); hyperplasia, melanocyte (0/50, 0/50, 0/50, 19/50); inflammation, chronic (13/50, 17/50, 26/50, 43/50)	Skin (site of application): epidermis, hyperplasia (7/50, 7/50, 15/50, 34/50); hyperplasia, melanocyte (1/50, 1/50, 3/50, 33/50); inflammation, chronic (37/50, 36/50, 43/50, 48/50)
Neoplastic effects	None	None	None	Liver: hepatoblastoma (0/50, 4/50, 0/50, 3/50); hepatocholangiocarcinoma (0/50, 0/50, 1/50, 2/50) Uterus: stromal polyp or stromal sarcoma (0/50, 1/50, 2/50, 6/50)
Equivocal Findings	Malignant mesothelioma: (0/50, 2/50, 2/50, 5/50)	None	None	None
Level of evidence of carcinogenic activity	Equivocal evidence	No evidence	No evidence	Some evidence
Genetic toxicology Bacterial gene mutations:			Negative in *Salmonella typhimurium* strains TA98 and TA100 and *Escherichia coli* strain WP2 *uvrA*/pKM101, with or without S9	

RATS

- Survival: no effect

TABLE 5
Survival of Rats in the 2-Year Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control	0.3 mg/kg	1.0 mg/kg	3.0 mg/kg
Male				
Animals initially in study	65	65	65	65
2-Week interim evaluation[a]	5	5	5	5
13-Week interim evaluation[a]	5	5	5	5
12-Month interim evaluation[a]	5	5	5	5
Accidental death[a]	0	1	0	0
Moribund	19	24	19	16
Natural deaths	8	7	3	11
Animals surviving to study termination	23	18	28	23
Percent probability of survival at end of study[b]	46	37	56	46
Mean survival (days)[c]	560	512	552	550
Survival analysis[d]	P=0.626N	P=0.161	P=0.581N	P=1.000
Female				
Animals initially in study	65	65	65	65
2-Week interim evaluation[a]	5	5	5	5
13-Week interim evaluation[a]	5	5	5	5
12-Month interim evaluation[a]	5	5	5	5
Moribund	10	12	17	10
Natural deaths	13	7	9	8
Animals surviving to study termination	27	31	24[e]	32
Percent probability of survival at end of study	54	62	48	64
Mean survival (days)	565	576	546	556
Survival analysis	P=0.603N	P=0.394N	P=0.559	P=0.485N

[a] Censored from survival analyses
[b] Kaplan-Meier determinations
[c] Mean of all deaths (uncensored, censored, and terminal kill).
[d] The result of the life table trend test (Tarone, 1975) is in the vehicle control column, and the results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the dosed group columns. A negative trend or a lower mortality in a dose group is indicated by N.
[e] Includes one animal that died during the last week of the study

- *Body weight and clinical signs*: Mean body weights of all dosed groups of male and female rats were within 10% of those of the vehicle control groups throughout the study. No chemical-related clinical findings were observed.

TABLE 6
Mean Body Weights and Survival of Male Rats in the 2-Year Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control		0.3 mg/kg			1.0 mg/kg			3.0 mg/kg		
Day	Av. Wt. (g)	No. of Survivors	Av. Wt (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt (g)	Wt. (% of Controls)	No. of Survivors
Mean for Weeks											
1-13	239		237	99		239	100		235	98	
14-52	415		414	100		412	99		408	98	
53-101	498		501	101		491	99		489	99	

[a] Interim evaluations occurred during weeks 2, 13, and 53.

TABLE 7
Mean Body Weights and Survival of Female Rats in the 2-Year Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control		0.3 mg/kg			1.0 mg/kg			3.0 mg/kg		
Day	Av. Wt. (g)	No. of Survivors	Av. Wt (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt (g)	Wt. (% of Controls)	No. of Survivors
Mean for Weeks											
1-13	155		156	100		155	100		155	100	
14-52	245		244	100		245	100		242	99	
53-101	328		330	101		329	101		323	99	

- **Pathology and Statistical Analyses**

- *Malignant Mesothelioma*: In male rats, there was a positive trend in the incidences of malignant mesothelioma; the incidence in 3.0 mg/kg males was significantly greater than the vehicle control incidence and exceeded historical control ranges for dermal studies (all vehicles) and for all routes of administration by only one tumor. Microscopically, malignant mesotheliomas were papillary and consisted of one or more layers of neoplastic mesothelial cells covering pedunculated fibrovascular stalks. In all cases, they were present in the tunics around the testes with dissemination into the peritoneal cavity. Mesotheliomas arising from vaginal tunics have been reported occasionally as spontaneous tumors in F344/N rats.
- *Skin*: Nonneoplastic skin lesions in the core study rats at the site of application included epidermal hyperplasia and hyperkeratosis. The incidences of these lesions in the male rats administered 1.0 or 3.0 mg/kg of trimethylolpropane triacrylate were significantly increased. In the females at the site of application, the incidences of epidermal hyperplasia were significantly increased at 1.0 and 3.0 mg/kg, and the incidences of hyperkeratosis were significantly increased in all dosed groups. Microscopically, epidermal hyperplasia was defined as increased cell layers in the epidermis and was considered minimal when the epidermis was thickened to three to four cell layers and was considered mild when there was a thickening of the epidermis to five to six cell layers. Hyperkeratosis was defined as thickening of the stratum corneum with concurrent expansion of the stratum granulosum, and the hyperkeratosis was considered minimal if the stratum corneum layer was slightly thickened by a thin amount of loosely packed keratin and was considered mild when thickened by a dense, compact band of keratin. The following neoplasms were identified in the core study males, as trace gross lesions remote from the site of application. Compared to the vehicle controls, the male rats administered 0.3 mg/kg had increases in the incidences of basal cell adenoma (0/50, 3/50, 0/50, 2/50), basal cell carcinoma (0/50, 2/50, 1/50, 0/50), and basal cell adenoma or basal cell carcinoma (combined) (0/50, 5/50, 1/50, 2/50). The incidence of basal cell adenoma or basal cell carcinoma (combined) was statistically significant in the 0.3 mg/kg group and exceeded the historical control range for all routes of study (0%-8%), but only by a single neoplasm. The

increased incidence of basal cell adenoma or basal cell carcinoma (combined) was statistically significant in the 0.3 mg/kg group, but again, this is the lowest dose group. Additionally, there was no dose relationship for these lesions, the incidences of basal cell carcinoma alone were not statistically significant and did not exceed the historical control range for all routes of study (0%-6%), and none of these lesions were at the site of application. Therefore, the increased incidences of these lesions compared to concurrent vehicle controls were not considered related to treatment. Interim evaluations of the proliferative skin effects of dermal exposure to trimethylolpropane triacrylate were conducted during weeks 2 and 13 and month 12. Histopathologic evaluation of the skin from the site of application identified treatmentrelated increases in the incidences of epidermal hyperplasia at all three time points in both sexes. There were also treatment-related increases in the incidences of sebaceous gland hyperplasia at 2 and 13 weeks in the male rats, and at 13 weeks in the female rats. At 12 months, there were treatment-related increases in the incidences of hyperkeratosis in both sexes. The dosed rats had up to five layers of epidermal cells (vehicle control rats had one to two layers). Sebaceous gland hyperplasia was characterized by a slight increase in the size of the sebaceous glands relative to the vehicle controls. Hyperkeratosis was characterized by an increase in the amount of keratin on the epidermal surface. At all three time points, these changes were considered to be minor, but the increases in the incidences of these lesions were clearly dose related.

TABLE 8
Incidences of Malignant Mesothelioma in Male Rats in the 2-Year Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control	0.3 mg/kg	1.0 mg/kg	3.0 mg/kg
Overall rate[a,b]	0/50 (0%)	2/50 (4%)	2/50 (4%)	5/50 (10%)
Adjusted rate[c]	0.0%	5.7%	4.9%	11.8%
Terminal rate[d]	0/23 (0%)	1/18 (6%)	1/28 (4%)	1/23 (4%)
First incidence (days)	—[f]	529	728	591
Poly-3 test[e]	P=0.024	P=0.201	P=0.231	P=0.031

[a] Number of animals with malignant mesothelioma per number necropsied
[b] Historical incidence for 2-year dermal study vehicle controls (all vehicles) (mean ± standard deviation): 8/250 (3.2% ± 3.4%), range 0%-8%; all routes: 40/1,249 (3.2% ± 2.8%), range 0%-8%
[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.
[f] Not applicable; no neoplasms in animal group

TABLE 9
Incidences of Nonneoplastic Lesions of the Skin at the Site of Application in Core Study Rats in the 2-Year Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control	0.3 mg/kg	1.0 mg/kg	3.0 mg/kg
Male				
Number Examined Microscopically	50	49	50	50
Epidermis, Hyperplasia[a]	1 (1.0)[b]	0	12** (1.0)	28** (1.1)
Hyperkeratosis	2 (1.0)	4 (1.0)	33** (1.0)	49** (1.0)
Female				
Number Examined Microscopically	50	50	50	50
Epidermis, Hyperplasia	0	4 (1.3)	11** (1.0)	25** (1.0)
Hyperkeratosis	0	11** (1.0)	42** (1.0)	50** (1.0)

** Significantly different (P≤0.01) from the vehicle control group by the Poly-3 test
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

MICE

- *Survival*: no effect

- *Body Weights and Clinical Findings*: Mean body weights of all dosed groups of male and female mice were within 10% of those of the vehicle control groups throughout the study. No chemical-related clinical findings were observed.

TABLE 10
Survival of Mice in the 2-Year Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control	0.3 mg/kg	1.0 mg/kg	3.0 mg/kg
Male				
Animals initially in study	65	65	65	65
2-Week interim evaluation[a]	5	5	5	5
13-Week interim evaluation[a]	5	5	5	5
12-Month interim evaluation[a]	5	5	5	5
Moribund	8	7	10	4
Natural deaths	12	8	11	8
Animals surviving to study termination	30	35	29	38
Percent probability of survival at end of study[b]	60	70	58	76
Mean survival (days)[c]	665	687	688	700
Survival analysis[d]	P=0.159N	P=0.387N	P=1.000	P=0.118N
Female				
Animals initially in study	65	65	65	65
2-Week interim evaluation[a]	5	5	5	5
13-Week interim evaluation[a]	5	5	5	5
12-Month interim evaluation[a]	5	5	5	5
Accidental death[a]	0	1	0	0
Moribund	8	7	8	7
Natural deaths	3	11	12	13
Animals surviving to study termination	39	31	30[e]	30
Percent probability of survival at end of study	78	63	60	60
Mean survival (days)	710	682	685	693
Survival analysis	P=0.234	P=0.159	P=0.073	P=0.094

[a] Censored from survival analyses
[b] Kaplan-Meier determinations
[c] Mean of all deaths (uncensored, censored, and terminal kill).
[d] The result of the life table trend test (Tarone, 1975) is in the vehicle control column, and the results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the dosed group columns. A negative trend or a lower mortality in a dose group is indicated by N.
[e] Includes one animal that died during the last week of the study

TABLE 11
Mean Body Weights and Survival of Male Mice in the 2-Year Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control		0.3 mg/kg			1.0 mg/kg			3.0 mg/kg		
Day	Av. Wt. (g)	No. of Survivors	Av. Wt (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt (g)	Wt. (% of Controls)	No. of Survivors
Mean for Weeks											
1-13	30.0		29.8	99		29.6	98		30.1	100	
14-52	49.2		49.4	100		48.8	99		49.6	101	
53-101	53.7		54.8	102		53.3	99		54.2	101	

[a] Interim evaluations occurred during weeks 2, 13, and 53.

TABLE 12
Mean Body Weights and Survival of Female Mice in the 2-Year Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control		0.3 mg/kg			1.0 mg/kg			3.0 mg/kg		
Day	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	Av. Wt. (g)	Wt. (% of Controls)	Av. Wt. (g)	Wt. (% of Controls)	Wt. (% of Controls)	Day	Av. Wt. (g)
Mean for Weeks											
1-13	25.1		25.6	102		25.2	101		25.6		102
14-52	49.2		51.0	104		50.5	103		50.6		103
53-101	62.8		64.3	102		63.6	101		63.0		100

[a] Interim evaluations occurred during weeks 2, 13, and 53.

- **Pathology and Statistical Analyses**

- *Liver:* Although not significant, there were increased incidences of hepatoblastoma in the 0.3 and 3.0 mg/kg groups and hepatocholangiocarcinoma in the 1.0 and 3.0 mg/kg groups of females. The historical control ranges for hepatoblastoma are low, while hepatocholangiocarcinoma has not been seen in historical controls. Based on the rarity of these neoplasms in female mice and their absence in the concurrent vehicle controls, hepatoblastoma and hepatocholangiocarcinoma were considered to be treatment-related lesions. Female mice exposed to trimethylolpropane triacrylate showed a positive trend in the incidences of hepatocellular carcinoma. However, increased incidences in treated groups were not significant and not dose related; therefore, this neoplasm is not considered treatment related. The incidences of eosinophilic focus (vehicle control, 15/50; 0.3 mg/kg, 23/50; 1.0 mg/kg, 21/50; 3.0 mg/kg, 21/50) in 0.3 mg/kg females and Kupffer cell pigmentation (4/50, 5/50, 10/50, 4/50) in 1.0 mg/kg females were significantly greater than those in the vehicle controls. The relationship of these nonneoplastic lesions to trimethylolpropane triacrylate administration is uncertain. Microscopically, hepatoblastomas were noted as irregular masses with blood-filled cystic spaces, necrosis, and hemorrhage, and they generally had scant stroma and were composed of small, deeply basophilic cells with scant cytoplasm and elongated nuclei (hepatoblasts). Hepatocholangiocarcinomas were recorded as a pattern similar to hepatocellular carcinomas with small areas of ducts often containing both neoplastic hepatocytes and epithelial cells often inconspicuous in the primary tumor and more obvious in metastases, and containing large necrotic or cystic areas within tumors. Hepatocellular carcinomas were noted to have trabecular patterns, with trabeculae at least three cells thick.

TABLE 13
Incidences of Neoplasms of the Liver in Female Mice in the 2-Year Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control	0.3 mg/kg	1.0 mg/kg	3.0 mg/kg
Number Examined Microscopically	50	50	50	50
Hepatoblastoma, Multiple[a]	0	1	0	0
Hepatoblastoma, (includes multiple)[b]	0	4	0	3
Hepatocholangiocarcinoma[c]	0	0	1	2
Hepatocellular Carcinoma, Multiple	3	3	5	2
Hepatocellular Carcinoma (includes multiple)[d]				
Overall rate[e]	12/50 (24%)	13/50 (26%)	10/50 (20%)	19/50 (38%)
Adjusted rate[f]	25.4%	28.4%	22.7%	41.3%
Terminal rate[g]	10/39 (26%)	6/31 (19%)	7/30 (23%)	12/30 (40%)
First incidence (days)	638	513	440	599
Poly-3 test[h]	P=0.045	P=0.461	P=0.479N	P=0.076

[a] Number of animals with neoplasm
[b] Historical incidence for 2-year dermal study vehicle controls (all vehicles) (mean ± standard deviation): 2/250 (0.8% ± 1.1%), range: 0%-2%; all routes: 4/1,195 (0.3% ± 0.8%), range: 0%-2%
[c] Historical incidence for 2-year dermal studies: 0/250; all routes: 0/1,195
[d] Historical incidence for 2-year dermal studies: 63/250 (25.2% ± 15.5%), range: 6%-46%; all routes: 144/1,195 (12.1% ± 10.8%), range: 0%-46%
[e] Number of animals with neoplasm per number of animals with liver examined microscopically
[f] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[g] Observed incidence at terminal kill
[h] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A lower incidence in a dose group is indicated by N.

- *Uterus:* The incidences of uterine stromal polyp and stromal polyp or stromal sarcoma (combined) in the female mice showed positive trends as well as significant increases in the 3.0 mg/kg group; the incidences in the 3.0 mg/kg females also exceeded the ranges in historical controls from dermal studies (all vehicles) and from all routes of administration. In addition, there was one female in the vehicle control group with a uterine sarcoma of uncertain orgin. Since the origin of the uterine sarcoma was uncertain, the diagnosis of uterine sarcoma was not included with the other uterine tumors. Microscopically, stromal polyps were pedunculated masses that protruded into the lumen, were often lined by cuboidal to columnar epithelium, and were composed of abundant amounts of loose stroma containing stellate or spindle cells, numerous blood vessels, and ectatic endometrial glands. Stromal sarcomas were distinguished from stromal polyps by marked cellular pleomorphism.

TABLE 14
Incidences of Neoplasms of the Uterus in Female Mice in the 2-Year Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control	0.3 mg/kg	1.0 mg/kg	3.0 mg/kg
Number Necropsied	50	50	50	50
Stromal Polyp[a]				
Overall rate[b]	0/50 (0%)	1/50 (2%)	2/50 (4%)	5/50 (10%)
Adjusted rate[c]	0.0%	2.3%	4.7%	11.1%
Terminal rate[d]	0/39 (0%)	1/31 (3%)	2/30 (7%)	4/30 (13%)
First incidence (days)	—[f]	729 (T)	729 (T)	409
Poly-3 test[e]	P=0.008	P=0.486	P=0.219	P=0.027
Stromal Sarcoma[g]	0	0	0	1
Stromal Polyp or Stromal Sarcoma[h]				
Overall rate	0/50 (0%)	1/50 (2%)	2/50 (4%)	6/50 (12%)
Adjusted rate	0.0%	2.3%	4.7%	13.3%
Terminal rate	0/39 (0%)	1/31 (3%)	2/30 (7%)	4/30 (13%)
First incidence (days)	—	729 (T)	729 (T)	409
Poly-3 test	P=0.002	P=0.486	P=0.219	P=0.014

(T) Terminal kill
[a] Historical incidence for 2-year dermal study vehicle controls (all vehicles) (mean ± standard deviation): 5/250 (2.0% ± 2.5%), range 0%-6%; all routes: 24/1,198 (2.0% ± 2.2%), range 0%–8%
[b] Number of animals with neoplasm per number of animals necropsied
[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.
[f] Not applicable; no neoplasms in animal group
[g] Historical incidence for 2-year dermal studies: 0/250; all routes: 2/1,198 (0.2% ± 0.6%), range 0%-2%
[h] Historical incidence for 2-year dermal studies: 5/250 (2.0% ± 2.5%), range 0%-6%; all routes: 26/1,198 (2.2% ± 2.2%), range 0%-8%

- *Skin (Site of Application):* Compared to the vehicle control incidences, the incidences of epidermal hyperplasia, melanocyte hyperplasia, and chronic inflammation were significantly increased in core study males and females administered 3.0 mg/kg; the incidences of epidermal hyperplasia in 1.0 mg/kg females and chronic inflammation in 1.0 mg/kg males were also significantly increased. Microscopically, epidermal hyperplasia consisted of a slight increase in the number of cell layers of the epidermis. Melanocyte hyperplasia was characterized by aggregates of cells containing dark brown granules of pigment, which were typically found in the superficial dermis. Chronic inflammation was characterized predominantly by mononuclear cells, with occasional neutrophils in the dermis. Acute inflammation, which was much less common than chronic inflammation, consisted mainly of neutrophils with fewer macrophages and lymphocytes in the dermis and epidermis adjacent to an eroded or ulcerated surface epithelium. Degenerate neutrophils, cell debris and necrotic epidermal cells, and proteinaceous fluid frequently formed a crust overlying the eroded or ulcerated epidermis. Interim evaluations of the proliferative skin effects of dermal exposure to trimethylolpropane triacrylate were conducted during weeks 2 and 13 and month 12. Histopathologic evaluation of the skin from the site of application identified slight treatment-related increases in the incidences of hyperplasia and inflammation or chronic active inflammation at all three time points in both sexes. Additionally, there were treatment-related increases in the incidences of sebaceous gland hyperplasia at the 13-week time point in both sexes. The dosed mice had up to five layers of epidermal cells (vehicle control mice had one to two layers). Sebaceous gland hyperplasia was characterized by a slight increase in the size of the sebaceous glands relative to the vehicle controls. The inflammation consisted of infiltrates of predominantly lymphocytes, with occasional macrophages, and the chronic active inflammation was characterized by infiltrates of predominantly macrophages with fewer neutrophils. The inflammatory cells were scattered throughout the dermis, multifocally in minimal inflammation, and more diffusely in mild and moderate inflammation. At all three time points, these changes were considered to be minor, but the increases in the incidences of these lesions were clearly dose related.

TABLE 15
Incidences of Nonneoplastic Lesions of the Skin at the Site of Application in Core Study Mice in the 2-Year Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control	0.3 mg/kg	1.0 mg/kg	3.0 mg/kg
Male				
Number Examined Microscopically	50	50	50	50
Epidermis, Hyperplasia[a]	10 (1.3)[b]	7 (1.9)	15 (1.2)	44** (1.7)
Hyperplasia, Melanocyte	0	0	0	19** (1.1)
Inflammation, Chronic	13 (1.2)	17 (1.1)	26** (1.1)	43** (1.3)
Female				
Number Examined Microscopically	50	50	50	50
Epidermis, Hyperplasia	7 (1.9)	7 (1.6)	15* (1.5)	34** (1.7)
Hyperplasia, Melanocyte	1 (1.0)	1 (4.0)	3 (1.7)	33** (1.3)
Inflammation, Chronic	37 (1.1)	36 (1.2)	43 (1.2)	48** (1.5)
Ulcer	0	0	3 (3.3)	3 (3.3)
Inflammation, Acute	1 (2.0)	1 (3.0)	2 (2.5)	4 (1.5)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Poly-3 test
** $P \leq 0.01$
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

- *Adrenal Medulla:* In male mice, there was a positive rend in the incidences of hyperplasia in the adrenal medulla (1/49, 4/49, 3/46, 10/50). The incidence in 3.0 mg/kg males was significantly greater than that in the vehicle controls and exceeded the historical range (0%-8%) in concurrent NTP studies by all routes. Microscopically, the lesion was focal, characterized by a circumscribed increase in the number and tinctorial basophilia of the chromaffin cells.

- *Other Neoplastic Findings:* In male mice administered 1.0 mg/kg trimethylolpropane triacrylate, there was a significant increase in the incidence of alveolar/ bronchiolar adenoma (1/50, 6/50, 10/50, 4/50); however, the incidence of alveolar/bronchiolar carcinoma (12/50, 11/50, 3/50, 10/50) in this group was significantly decreased. The increased incidence of alveolar/bronchiolar adenoma was not considered to be a treatment-related effect due to the absence of a significant positive trend, because the combined incidences of adenoma or carcinoma (13/50, 17/50, 13/50, 14/50) were not significantly increased at any dose, and the incidences were within the historical control ranges for all routes of study [alveolar/ bronchiolar adenoma: 172/1,150 (15% ± 7%), range 2%-30%; alveolar/bronchiolar carcinoma: 144/1,150 (13% ± 7%), range 4%-24%; alveolar/bronchiolar adenoma or carcinoma (combined): 301/1,150 (26% ± 6%), range 14%-40%].

- *Other Nonneoplastic Findings:* In male mice administered 3.0 mg/kg, there was a significantly increased incidence of mineralization in the glandular stomach (1/48, 3/49, 2/44, 8/49; Table C3). This was considered to be a common background lesion, and the increased incidence was considered to be sporadic and most likely unrelated to trimethylolpropane triacrylate administration.

1.3 Specific target organ toxicity-repeated exposure

1.3.1 Animal data

1.3.1.1 [NTP, 2005]

Study reference:

NTP (2005). NTP report on the toxicology studies of trimethylolpropane triacrylate (technical grade) (CAS No. 15625-89-5) in F344/N rats, B6C3F1 mice and genetically modified (FVB Tg. Ac hemizygous) mice (dermal studies).

Detailed study summary and results:

Test type

Not guideline; GLP compliant

Test substance

- TMPTA
- Degree of purity = 80%
- GC indicated one major peak and two impurities with areas of 6.5% and 3.4% relative to the major peak area. HPLC indicated a major peak and five impurities with a combined area of 22.2%. HPLC/MS indicated 10 impurities contributing or corresponding to the impurity peaks identified by HPLC. These impurities included four structurally related acrylates or adducts: trimethylolpropane diacrylate, trimethylolpropane triacrylate acrylic acid adduct, trimethylolpropane triacrylate-trimethylolpropane monoacrylate adduct, and trimethylolpropane triacrylatetrimethylolpropane diacrylate adduct. No substantial amount of 4-methoxyphenol, a stabilizer added to trimethylolpropane triacrylate, was detected.
- Batch number: 01031AW

Test animals

- F344/N rats
- B6C3Fmice
- 5/sex/group

Administration/exposure

- Application on the backs of male and female 5 days per week for 16 days.
- 0, 12.5, 25, 50, 100, or 200 mg/kg
- Animals painted with acetone alone served as the control groups.
- Skin was examined for every animal and thymus for every mice.

TABLE 1
Experimental Design and Materials and Methods in the Dermal Studies of Trimethylolpropane Triacrylate

2-Week Studies	3-Month Studies	6-Month Study
Study Laboratory Battelle Columbus Laboratories (Columbus, OH)	Battelle Columbus Laboratories (Columbus, OH)	Battelle Columbus Laboratories (Columbus, OH)
Strain and Species F344/N rats $B6C3F_1$ mice	F344/N rats $B6C3F_1$ mice	Tg.AC [FVB/N-TgN(V-Ha-*ras*)] hemizygous mice
Animal Source Taconic Laboratory Animals and Services (Germantown, NY)	Taconic Laboratory Animals and Services (Germantown, NY)	Taconic Laboratory Animals and Services (Germantown, NY)
Time Held Before Studies Rats: 11 days Mice: 12 days	Rats: 11 days (males) or 12 days (females) Mice: 13 days (females) or 14 days (males)	11 days
Average Age When Studies Began 6 weeks	6 weeks	6 weeks
Date of First Dose Rats: May 6, 1996 Mice: May 7, 1996	Rats: September 9 (male) or 10 (female), 1996 Mice: September 11 (female) or 12 (male), 1996	July 20, 1998
Duration of Dosing 5 days per week for 16 days	5 days per week for 14 weeks	Core study: 5 days per week for 28 weeks Positive control: 3 days per week for 28 weeks
Date of Last Dose Rats: May 21, 1996 Mice: May 22, 1996	Rats: December 10 (male) or 11 (female), 1996 Mice: December 12 (female) or 13 (male), 1996	Core study: January 27 (male) or 28 (female), 1999 Positive control: January 28, 1999
Necropsy Dates Rats: May 22, 1996 Mice: May 23, 1996	Rats: December 10 (male) or 11 (female), 1996 Mice: December 12 (female) or 13 (male), 1996	Core study: January 27 (male) or 28 (female), 1999 Positive control: January 28, 1999
Average Age at Necropsy 8 weeks	Rats: 19 weeks Mice: 19 (female) or 20 (male) weeks	33 weeks
Size of Study Groups 5 males and 5 females	10 males and 10 females	15 males and 15 females
Method of Distribution Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 2-week studies	Same as 2-week studies
Animals per Cage 1	1	1

TABLE 1
Experimental Design and Materials and Methods in the Dermal Studies of Trimethylolpropane Triacrylate

2-Week Studies	3-Month Studies	6-Month Study
Method of Animal Identification Tail tattoo	Tail tattoo	Tail tattoo
Diet NTP-2000 pelleted diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, changed weekly	Same as 2-week studies, except feed was irradiated	Same as 3-month studies
Water Tap water (Columbus municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available *ad libitum*	Same as 2-week studies	Same as 2-week studies
Cages Polycarbonate (Lab Products, Inc., Maywood, NJ), changed at least once per week, rotated every 2 weeks	Same as 2-week studies	Same as 2-week studies
Bedding Sani-Chip® hardwood chips (P.J. Murphy Forest Products Corp., Montville, NJ), changed at least once per week	Same as 2-week studies, except bedding was irradiated	Same as 3-month studies
Cage Filters Spun-bonded DuPont 2024 polyester (Snow Filtration Co., Cincinnati, OH), changed every 2 weeks	Same as 2-week studies	Same as 2-week studies
Racks Stainless steel, cleaned and rotated every 2 weeks	Same as 2-week studies	Same as 2-week studies
Animal Room Environment Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour
Doses 0, 12.5, 25, 50, 100, or 200 mg/kg in acetone by dermal application (dosing volume 0.5 mL/kg for rats and 2 mL/kg for mice)	0, 0.75, 1.5, 3, 6, or 12 mg/kg in acetone by dermal application (dosing volume 0.5 mL/kg for rats and 2 mL/kg for mice)	Core study: 0, 0.75, 1.5, 3, 6, or 12 mg/kg in acetone by dermal application (dosing volume 3.3 mL/kg) Positive control: 1.25 µg 12-*O*-tetradecanoylphorbol-13-acetate/100 mL acetone by dermal application (dosing volume 100 µL)
Type and Frequency of Observation Animals were observed twice daily and were weighed initially, on day 8, and at the end of the studies. Clinical findings were recorded daily.	Animals were observed twice daily and were weighed initially, weekly, and at the end of the studies. Clinical findings were recorded weekly for core study animals.	Animals were observed twice daily and were weighed initially, weekly, and at the end of the study. Clinical findings were recorded weekly and at the end of the study. Observations of papilloma formation on the skin were recorded weekly.

TABLE 1
Experimental Design and Materials and Methods in the Dermal Studies of Trimethylolpropane Triacrylate

2-Week Studies	3-Month Studies	6-Month Study
Method of Sacrifice Carbon dioxide asphyxiation	Same as 2-week studies	Same as 2-week studies
Necropsy Necropsies were performed on all animals. Organs weighed were the heart, right kidney, liver, lung, right testis, and thymus.	Necropsies were performed on all animals. Organs weighed were the heart, right kidney, liver, lung, right testis, and thymus.	Necropsies were performed on core study mice. Organs weighed were the heart, right kidney, liver, lung, right testis, and thymus.
Clinical Pathology None	Blood was collected from the retroorbital sinus of special study rats on days 4 and 23 and from core study rats and mice at the end of the studies for hematology and clinical chemistry (rats only). ***Hematology:*** hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, and platelet counts; erythrocyte morphology; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials ***Clinical chemistry:*** urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and bile acids	None
Histopathology Histopathologic examinations were performed on the skin (site of application) of all animals and the thymus of all mice.	Complete histopathologic examinations were performed on vehicle control and 12 mg/kg rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, gallbladder (mice), heart with aorta, large intestine (cecum, colon, and rectum), small intestine (duodenum, jejunum, and ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin (site of application), spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus. The skin at the site of application was also examined in the remaining core study groups.	Histopathologic examinations were performed on all core study mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, heart, kidney, liver, lung, lymph nodes (mandibular, mediastinal, and mesenteric), ovary, pituitary gland, skin (site of application and inguinal), spleen, stomach (forestomach), testis with epididymis, thymus, thyroid gland, and uterus.

Results and discussion

- Survival and body weight: no effect

TABLE 3
Incidences of Nonneoplastic Lesions of the Skin (Site of Application) in Rats in the 2-Week Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg
Male						
Number Examined						
Microscopically	5	5	5	5	5	5
Epidermis, Hyperplasia[a]	0	5** (1.4)[b]	5** (1.8)	5** (2.6)	5** (3.2)	5** (3.4)
Hyperkeratosis	0	5** (1.6)	5** (1.2)	5** (2.4)	5** (2.0)	5** (2.0)
Sebaceous Glands, Hyperplasia	0	5** (2.0)	5** (1.8)	5** (2.8)	5** (2.2)	5** (2.0)
Dermis, Inflammation, Chronic Active	0	5** (1.6)	5** (2.0)	5** (2.8)	5** (2.8)	5** (3.4)
Ulcer	0	1 (2.0)	1 (1.0)	5** (2.2)	5** (2.6)	5** (2.8)
Epidermis, Degeneration	0	4* (1.3)	4* (1.0)	5** (2.4)	5** (2.6)	5** (3.2)
Parakeratosis	0	1 (1.0)	4* (1.8)	5** (2.6)	5** (3.2)	5** (3.8)
Epidermis, Inflammation, Suppurative	0	2 (1.5)	5** (1.8)	5** (3.0)	5** (3.0)	5** (3.8)
Female						
Number Examined						
Microscopically	5	5	5	5	5	5
Epidermis, Hyperplasia	0	5** (1.2)	5** (2.0)	5** (2.6)	5** (3.2)	5** (3.6)
Hyperkeratosis	0	5** (1.0)	5** (1.4)	5** (1.2)	5** (2.0)	3* (2.0)
Sebaceous Glands, Hyperplasia	0	5** (1.4)	5** (2.0)	5** (2.2)	5** (2.2)	5** (1.8)
Dermis, Inflammation, Chronic Active	0	3* (1.0)	5** (2.0)	5** (2.8)	5** (3.0)	5** (3.4)
Ulcer	0	0	4* (1.5)	5** (3.2)	5** (2.6)	5** (3.0)
Epidermis, Degeneration	0	1 (1.0)	5** (2.2)	5** (3.0)	5** (3.0)	5** (3.0)
Parakeratosis	0	3* (1.7)	5** (2.2)	5** (3.0)	5** (3.0)	5** (3.2)
Epidermis, Inflammation, Suppurative	0	1 (1.0)	4* (2.0)	5** (3.0)	5** (3.4)	5** (3.2)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test
** $P \leq 0.01$
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

TABLE 7
Incidences of Selected Nonneoplastic Lesions in B6C3F$_1$ Mice in the 2-Week Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg
Male						
Skin, Site of Application[a]	5	5	5	5	5	5
Epidermis, Hyperplasia[b]	0	5** (1.6)[c]	5** (2.2)	5** (2.2)	5** (2.8)	5** (3.6)
Hyperkeratosis	0	5** (1.4)	4* (1.3)	5** (1.8)	3* (1.7)	3* (1.7)
Dermis, Inflammation, Chronic Active	0	5** (1.8)	5** (1.8)	5** (2.6)	5** (3.0)	5** (2.8)
Sebaceous Gland, Hyperplasia	0	5** (2.2)	5** (1.6)	2 (2.5)	5** (1.6)	1 (1.0)
Ulcer	0	0	0	2 (1.0)	2 (1.0)	5** (2.8)
Epidermis, Degeneration	0	3* (1.0)	4* (1.0)	1 (1.0)	1 (1.0)	1 (1.0)
Parakeratosis	0	0	0	2 (2.0)	4* (2.3)	5** (2.4)
Epidermis, Inflammation, Suppurative	0	5** (1.4)	2 (1.0)	4* (1.8)	4* (1.8)	5** (3.0)
Thymus	5	5	5	5	5	5
Atrophy	0	0	0	0	3* (2.0)	3* (2.0)
Female						
Skin, Site of Application	5	5	5	5	5	5
Epidermis, Hyperplasia	0	5** (1.6)	5** (1.8)	5** (2.2)	5** (2.8)	5** (3.0)
Hyperkeratosis	0	3* (1.0)	4* (1.5)	5** (1.8)	4** (1.8)	4* (1.8)
Dermis, Inflammation, Chronic Active	0	5** (2.2)	5** (2.2)	5** (2.0)	5** (2.4)	5** (2.8)
Sebaceous Gland, Hyperplasia	0	5** (2.8)	5** (2.4)	5** (2.2)	5** (2.2)	5** (2.2)
Ulcer	0	0	1 (1.0)	0	3* (2.3)	3* (1.3)
Epidermis, Degeneration	0	2 (1.0)	3* (1.3)	1 (1.0)	3* (1.3)	4* (1.5)
Parakeratosis	0	1 (2.0)	2 (1.0)	1 (2.0)	3* (1.7)	5** (2.0)
Epidermis, Inflammation, Suppurative	0	1 (1.0)	4* (1.5)	2 (1.0)	5** (2.0)	5** (2.0)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test
** $P \leq 0.01$
[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

1.3.1.2 [NTP, 2005]

Study reference:

NTP (2005). NTP report on the toxicology studies of trimethylolpropane triacrylate (technical grade) (CAS No. 15625-89-5) in F344/N rats, B6C3F1 mice and genetically modified (FVB Tg. Ac hemizygous) mice (dermal studies).

Detailed study summary and results:

Test type

Not guideline; GLP compliant

Test substance

- TMPTA
- Degree of purity = 80%

- GC indicated one major peak and two impurities with areas of 6.5% and 3.4% relative to the major peak area. HPLC indicated a major peak and five impurities with a combined area of 22.2%. HPLC/MS indicated 10 impurities contributing or corresponding to the impurity peaks identified by HPLC. These impurities included four structurally related acrylates or adducts: trimethylolpropane diacrylate, trimethylolpropane triacrylate acrylic acid adduct, trimethylolpropane triacrylate-trimethylolpropane monoacrylate adduct, and trimethylolpropane triacrylatetrimethylolpropane diacrylate adduct. No substantial amount of 4-methoxyphenol, a stabilizer added to trimethylolpropane triacrylate, was detected.
- Batch number: 01031AW

Test animals

- F344/N rats
- B6C3Fmice
- 10/sex/group

Administration/exposure

- Application on the backs of male and female 5 days per week for 14 weeks.
- 0, 0.75, 1.5, 3, 6, or 12mg/kg
- Animals painted with acetone alone served as the control groups.
- Complete histopathologic examinations.

TABLE 1
Experimental Design and Materials and Methods in the Dermal Studies of Trimethylolpropane Triacrylate

2-Week Studies	3-Month Studies	6-Month Study
Study Laboratory Battelle Columbus Laboratories (Columbus, OH)	Battelle Columbus Laboratories (Columbus, OH)	Battelle Columbus Laboratories (Columbus, OH)
Strain and Species F344/N rats B6C3F$_1$ mice	F344/N rats B6C3F$_1$ mice	Tg.AC [FVB/N-TgN(V-Ha-*ras*)] hemizygous mice
Animal Source Taconic Laboratory Animals and Services (Germantown, NY)	Taconic Laboratory Animals and Services (Germantown, NY)	Taconic Laboratory Animals and Services (Germantown, NY)
Time Held Before Studies Rats: 11 days Mice: 12 days	Rats: 11 days (males) or 12 days (females) Mice: 13 days (females) or 14 days (males)	11 days
Average Age When Studies Began 6 weeks	6 weeks	6 weeks
Date of First Dose Rats: May 6, 1996 Mice: May 7, 1996	Rats: September 9 (male) or 10 (female), 1996 Mice: September 11 (female) or 12 (male), 1996	July 20, 1998
Duration of Dosing 5 days per week for 16 days	5 days per week for 14 weeks	Core study: 5 days per week for 28 weeks Positive control: 3 days per week for 28 weeks
Date of Last Dose Rats: May 21, 1996 Mice: May 22, 1996	Rats: December 10 (male) or 11 (female), 1996 Mice: December 12 (female) or 13 (male), 1996	Core study: January 27 (male) or 28 (female), 1999 Positive control: January 28, 1999
Necropsy Dates Rats: May 22, 1996 Mice: May 23, 1996	Rats: December 10 (male) or 11 (female), 1996 Mice: December 12 (female) or 13 (male), 1996	Core study: January 27 (male) or 28 (female), 1999 Positive control: January 28, 1999
Average Age at Necropsy 8 weeks	Rats: 19 weeks Mice: 19 (female) or 20 (male) weeks	33 weeks
Size of Study Groups 5 males and 5 females	10 males and 10 females	15 males and 15 females
Method of Distribution Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 2-week studies	Same as 2-week studies
Animals per Cage 1	1	1

TABLE 1
Experimental Design and Materials and Methods in the Dermal Studies of Trimethylolpropane Triacrylate

2-Week Studies	3-Month Studies	6-Month Study
Method of Animal Identification Tail tattoo	Tail tattoo	Tail tattoo
Diet NTP-2000 pelleted diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, changed weekly	Same as 2-week studies, except feed was irradiated	Same as 3-month studies
Water Tap water (Columbus municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available *ad libitum*	Same as 2-week studies	Same as 2-week studies
Cages Polycarbonate (Lab Products, Inc., Maywood, NJ), changed at least once per week, rotated every 2 weeks	Same as 2-week studies	Same as 2-week studies
Bedding Sani-Chip® hardwood chips (P.J. Murphy Forest Products Corp., Montville, NJ), changed at least once per week	Same as 2-week studies, except bedding was irradiated	Same as 3-month studies
Cage Filters Spun-bonded DuPont 2024 polyester (Snow Filtration Co., Cincinnati, OH), changed every 2 weeks	Same as 2-week studies	Same as 2-week studies
Racks Stainless steel, cleaned and rotated every 2 weeks	Same as 2-week studies	Same as 2-week studies
Animal Room Environment Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour
Doses 0, 12.5, 25, 50, 100, or 200 mg/kg in acetone by dermal application (dosing volume 0.5 mL/kg for rats and 2 mL/kg for mice)	0, 0.75, 1.5, 3, 6, or 12 mg/kg in acetone by dermal application (dosing volume 0.5 mL/kg for rats and 2 mL/kg for mice)	Core study: 0, 0.75, 1.5, 3, 6, or 12 mg/kg in acetone by dermal application (dosing volume 3.3 mL/kg) Positive control: 1.25 µg 12-*O*-tetradecanoylphorbol-13-acetate/100 mL acetone by dermal application (dosing volume 100 µL)
Type and Frequency of Observation Animals were observed twice daily and were weighed initially, on day 8, and at the end of the studies. Clinical findings were recorded daily.	Animals were observed twice daily and were weighed initially, weekly, and at the end of the studies. Clinical findings were recorded weekly for core study animals.	Animals were observed twice daily and were weighed initially, weekly, and at the end of the study. Clinical findings were recorded weekly and at the end of the study. Observations of papilloma formation on the skin were recorded weekly.

TABLE 1
Experimental Design and Materials and Methods in the Dermal Studies of Trimethylolpropane Triacrylate

2-Week Studies	3-Month Studies	6-Month Study
Method of Sacrifice Carbon dioxide asphyxiation	Same as 2-week studies	Same as 2-week studies
Necropsy Necropsies were performed on all animals. Organs weighed were the heart, right kidney, liver, lung, right testis, and thymus.	Necropsies were performed on all animals. Organs weighed were the heart, right kidney, liver, lung, right testis, and thymus.	Necropsies were performed on core study mice. Organs weighed were the heart, right kidney, liver, lung, right testis, and thymus.
Clinical Pathology None	Blood was collected from the retroorbital sinus of special study rats on days 4 and 23 and from core study rats and mice at the end of the studies for hematology and clinical chemistry (rats only). ***Hematology:*** hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, and platelet counts; erythrocyte morphology; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials ***Clinical chemistry:*** urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and bile acids	None
Histopathology Histopathologic examinations were performed on the skin (site of application) of all animals and the thymus of all mice.	Complete histopathologic examinations were performed on vehicle control and 12 mg/kg rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, gallbladder (mice), heart with aorta, large intestine (cecum, colon, and rectum), small intestine (duodenum, jejunum, and ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin (site of application), spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus. The skin at the site of application was also examined in the remaining core study groups.	Histopathologic examinations were performed on all core study mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, heart, kidney, liver, lung, lymph nodes (mandibular, mediastinal, and mesenteric), ovary, pituitary gland, skin (site of application and inguinal), spleen, stomach (forestomach), testis with epididymis, thymus, thyroid gland, and uterus.

TABLE 1
Experimental Design and Materials and Methods in the Dermal Studies of Trimethylolpropane Triacrylate

2-Week Studies	3-Month Studies	6-Month Study
Sperm Motility and Vaginal Cytology None	At the end of the studies, sperm samples were collected from male animals in the vehicle control and 3, 6, and 12 mg/kg groups for sperm count and motility evaluations. The following parameters were evaluated: spermatid heads per testis or cauda and per gram testis or cauda, and epididymal spermatozoal motility. The left cauda, left epididymis, and left testis were weighed. Vaginal samples were collected for up to 12 consecutive days prior to the end of the studies from females in the vehicle control and 3, 6, and 12 mg/kg groups for vaginal cytology evaluations. The percentage of time spent in the various estrous cycle stages and estrous cycle length were evaluated.	None

Results and discussion

- Survival and body weight: no effect

TABLE 4
Survival and Body Weights of Rats in the 3-Month Dermal Study of Trimethylolpropane Triacrylate

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g) Initial	Final	Change	Final Weight Relative to Controls (%)
Male					
0	10/10	88 ± 3	272 ± 6	183 ± 7	
0.75	10/10	89 ± 3	288 ± 6	199 ± 5	106
1.5	10/10	87 ± 3	270 ± 8	183 ± 9	99
3	10/10	88 ± 3	263 ± 5	174 ± 4	97
6	10/10	87 ± 3	267 ± 6	180 ± 6	98
12	10/10	89 ± 4	259 ± 7	170 ± 7	95
Female					
0	10/10	82 ± 2	171 ± 3	89 ± 3	
0.75	10/10	81 ± 2	164 ± 2	83 ± 3	96
1.5	10/10	83 ± 2	170 ± 2	87 ± 2	99
3	10/10	82 ± 2	173 ± 3	91 ± 3	101
6	10/10	82 ± 2	164 ± 2	81 ± 2	96
12	10/10	83 ± 2	169 ± 3	86 ± 4	99

[a] Number of animals surviving at 3 months/number initially in group
[b] Weights and weight changes are given as mean ± standard error.

TABLE 8
Survival and Body Weights of B6C3F$_1$ Mice in the 3-Month Dermal Study of Trimethylolpropane Triacrylate

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g) Initial	Final	Change	Final Weight Relative to Controls (%)
Male					
0	10/10	22.6 ± 0.4	36.5 ± 0.7	13.9 ± 0.6	
0.75	10/10	22.5 ± 0.4	34.5 ± 0.7	12.0 ± 0.5	95
1.5	10/10	22.2 ± 0.4	35.6 ± 1.1	13.4 ± 0.8	98
3	10/10	22.4 ± 0.5	35.8 ± 0.8	13.4 ± 0.9	98
6	10/10	22.3 ± 0.4	34.2 ± 0.7	11.9 ± 0.4	94
12	10/10	22.6 ± 0.5	34.6 ± 0.7	12.0 ± 0.9	95
Female					
0	10/10	19.4 ± 0.4	31.0 ± 1.2	11.6 ± 0.9	
0.75	10/10	19.6 ± 0.4	32.6 ± 0.7	13.0 ± 0.6	105
1.5	10/10	19.3 ± 0.4	31.9 ± 1.3	12.6 ± 1.2	103
3	10/10	19.1 ± 0.4	32.4 ± 1.2	13.3 ± 0.9	105
6	10/10	19.0 ± 0.4	30.1 ± 0.7	11.1 ± 0.6	97
12	10/10	19.3 ± 0.2	31.4 ± 1.1	12.2 ± 1.0	101

[a] Number of animals surviving at 3 months/number initially in group
[b] Weights and weight changes are given as mean ± standard error.

TABLE 5
Incidences of Selected Nonneoplastic Lesions of the Skin (Site of Application) in Rats in the 3-Month Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Male						
Numbered Examined						
Microscopically	10	10	10	10	10	10
Epidermis, Hyperplasia[a]	0	4* (1.0)[b]	7** (1.0)	10** (1.2)	10** (1.1)	10** (1.6)
Epidermis, Degeneration	0	0	4* (1.0)	7** (1.0)	9** (1.0)	8** (1.9)
Dermis, Inflammation, Chronic Active	0	1 (1.0)	3 (1.0)	6** (1.0)	10** (1.0)	10** (2.1)
Hyperkeratosis	0	0	5* (1.0)	10** (1.2)	10** (1.3)	10** (1.6)
Epidermis, Inflammation, Suppurative	0	0	0	0	0	4* (1.5)
Sebaceous Gland, Hyperplasia	0	0	5* (1.0)	10** (1.2)	10** (1.5)	10** (2.6)
Female						
Numbered Examined						
Microscopically	10	10	10	10	10	10
Epidermis, Hyperplasia	0	0	0	7** (1.0)	10** (1.1)	10** (1.4)
Epidermis, Degeneration	0	0	0	4* (1.0)	7** (1.0)	10** (2.0)
Epidermis, Necrosis	0	0	0	0	0	5* (2.0)
Dermis, Inflammation, Chronic Active	1 (1.0)	2 (1.0)	1 (1.0)	9** (1.0)	8** (1.0)	10** (1.8)
Hyperkeratosis	0	0	3 (1.0)	9** (1.0)	10** (1.1)	10** (2.0)
Epidermis, Inflammation, Suppurative	0	0	0	0	0	6** (1.3)
Sebaceous Gland, Hyperplasia	0	1 (1.0)	6** (1.0)	9** (1.1)	10** (1.3)	10** (2.1)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test
** $P \leq 0.01$
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

TABLE 9
Incidences of Selected Nonneoplastic Lesions of the Skin (Site of Application) in B6C3F$_1$ Mice in the 3-Month Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Male						
Number Examined						
Microscopically	10	10	10	10	10	10
Epidermis, Hyperplasia[a]	0	0	3 (1.0)[b]	10** (1.0)	10** (1.4)	10** (2.2)
Epidermis, Degeneration	0	0	0	4* (1.0)	8** (1.0)	9** (1.8)
Epidermis, Necrosis	0	0	1 (1.0)	1 (1.0)	2 (1.5)	7** (2.1)
Dermis, Inflammation, Chronic Active	1 (1.0)	0	2 (1.0)	10** (1.1)	9** (1.4)	10** (1.8)
Hyperkeratosis	0	0	3 (1.0)	8** (1.0)	8** (1.0)	10** (1.4)
Epidermis, Inflammation, Suppurative	0	0	1 (1.0)	0	1 (1.0)	8** (1.6)
Dermis, Fibrosis	0	0	0	0	0	7** (1.1)
Sebaceous Gland, Hyperplasia	0	0	0	9** (1.0)	10** (1.7)	10** (2.3)
Female						
Number Examined						
Microscopically	10	10	10	10	10	10
Epidermis, Hyperplasia	0	0	3 (1.0)	10** (1.0)	9** (1.1)	10** (1.3)
Epidermis, Degeneration	0	0	0	0	5* (1.0)	9** (1.6)
Epidermis, Necrosis	0	0	0	1 (1.0)	2 (2.0)	8** (2.0)
Dermis, Inflammation, Chronic Active	0	0	7** (1.0)	10** (1.1)	10** (2.0)	10** (1.8)
Hyperkeratosis	0	0	3 (1.0)	10** (1.0)	9** (1.0)	8** (1.1)
Epidermis, Inflammation, Suppurative	0	0	1 (1.0)	1 (1.0)	2 (1.5)	5* (1.4)
Dermis, Fibrosis	0	0	0	0	1 (1.0)	7** (1.6)
Sebaceous Gland, Hyperplasia	0	0	1 (1.0)	10** (1.0)	10** (2.0)	10** (2.3)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test
** $P \leq 0.01$
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

2 ENVIRONMENTAL HAZARDS

2.1 Degradation

2.1.1 Ready biodegradability (screening studies)

2.1.1.1 [Anonymous (2010)]

Study reference:

'Confidential Substance name' Ready Biodegradability - Evaluation of the Aerobic Biodegradability in an Aqueous Medium: OECD 301 B: CO2 Evolution Test.

Detailed study summary and results:

Test type:

OECD Guideline 301 B (Ready Biodegradability: CO2 Evolution Test)

EU Method C.4-C (Determination of the "Ready" Biodegradability - Carbon Dioxide Evolution Test)

GLP compliant

Test substance:

2,2-bis(prop-2-enoyloxymethyl)butyl prop-2-enoate / 15625-89-5 / 239-701-3

EC number 239-701-3

CAS number 15625-89-5

IUPAC name: 2,2-bis(prop-2-enoyloxymethyl)butyl prop-2-enoate

Batch Number: S700490021

Purity: 100% (active matter); known impurities: 5 - 10% tri-methylolpropane diacrylate and about 5% Michael adducts resulting from thermal self-condensation reactions

Materials and methods:

Oxygen conditions

aerobic

Inoculum or test system

activated sludge, domestic, non-adapted

Details on inoculum

- Source of inoculum/activated sludge (e.g. location, sampling depth, contamination history, procedu

re): Not adapted activated sludge from the aeration tank of the ARA Werdhölzli (8048 Zürich, Switze rland), a municipal biological waste water treatment plant.
Sampling: 23 March 2010, 11:00 a.m.
- Pretreatment: The activated sludge was used after sampling from the treatment plant without adaptation. However, the sludge was pre-conditioned for 2 days (aerated but not fed) to reduce the amount of CO2 produced by the blank controls.
Prior to the test the sludge was washed twice with tap water. After centrifugation the sludge, at a tenfold concentration of the final concentration to be achieved for the test, was suspended in test mediumas described in Table 1 (see below).
- Concentration of sludge: 30 mg/l dry matter in the final mixture

Duration of test (contact time): 28d

Initial test substance concentration : 33 mg/L test material, 20 mg/L based on TOC

Parameter followed for biodegradation estimation : CO2 evolution

Details on analytical methods
Inorganic carbon (IC) was determined with a Shimadzu TOC-5000A TOC-Analyzer (Shimadzu Schweiz GmbH, Römerstr. 3, CH-4153 Reinach) using the IC-mode. For each determination 3 single injections were performed.

Details on study design
TEST CONDITIONS
- Composition of medium: according to OECD guidelines
- Additional substrate: none
- Solubilising agent (type and concentration if used): none
- Test temperature: 22± 2 °C
- pH: 7.4± 0.2
- pH adjusted: yes (at beginning of test, with NaOH or HCl, if necessary)
- CEC (meq/100 g): no data
- Aeration of dilution water: aerated with CO2-free air
- Suspended solids concentration: 30 mg/l dry matter in the final mixture
- Continuous darkness: yes
- Other: Concentration of test substance and reference material: The test substance and reference material were applied by direct addition to give a final test concentration of about 20 mg/l with respect to the total organic carbon (TOC).

TEST SYSTEM

- Culturing apparatus: 2500 ml closed gas bottle
- Number of culture flasks/concentration: Test suspension containing inoculum, test medium and test substance (three replicates)
- Method used to create aerobic conditions: The test vessels were stirred thoroughly and aerated with synthetic CO2-free air
- Test performed in open system: yes
- Details of trap for CO2 and volatile organics if used: The air leaving the individual vessels was passed through gas-absorption bottles filled with KOH (125 ml of 0.13 M KOH).

SAMPLING

- Sampling frequency: The biodegradation of the test material was followed by CO2 measurements at frequent intervals to allow the assessment of the 10-d window (after 0, 1, 4, 7, 12, 14, 18, 21, 26, 28 days).
- Sampling method: not specified
- Sterility check if applicable: -
- Sample storage before analysis: -
- Other: -

CONTROL AND BLANK SYSTEM

- Inoculum blank: Inoculum blank containing inoculum and test medium (three replicates)
- Abiotic sterile control: Abiotic sterile control: containing test substance, test medium and 0.2 mM HgCl2 as sterilizing agent to prevent microbial decomposition (one replicate)
- Toxicity control: Toxicity control: containing inoculum + test medium + test substance + sodium benzoate as ready biodegradable reference compound (one replicate). In the toxicity control about 20 mg/l test substance and about 20 mg/l reference substance with respect to the total organic carbon (TOC) were tested as a mixture.
- Other: Procedure control: Procedure control containing inoculum, test medium and sodium benzoate as ready biodegradable reference compound (three replicates)

Reference substance

benzoic acid, sodium salt 100% pure

Results:

Parameter

% degradation (CO2 evolution)

% Degradation of test substance: 82 — 90 after 28 d (CO2 evolution) (range of 2 replicates)

Details on results

Based on the data of the individual inorganic carbon determinations, the mean biodegradability in the CO2 Evolution Test of the test substance was calculated to be 86% after 28 days. The biodegradation of the test

substance reached 66% at the end of the 10-d window. Significant biodegradation of the test substance was observed after a lag phase of about 7 days.

Results with reference substance

The positive control, sodium benzoate, reached 100% biodegradation after 14 days, thus confirming suitability of inoculum and test conditions.

Results of additional controls:

Toxicity control

At the applied initial test concentration of the test substance showed no significant toxic effect on the microbial population, since the biodegradation of the mixture (test substance + reference compound sodium benzoate) was within the expected theoretical value during thewhole test period. The test was considered valid, since more than 25 % degradation occurred within 14 days.

Abiotic sterile control. The test substance was not abiotically degraded (by processes producing CO2) during thewhole test period of 28 days in the absence of microorganisms as confirmed by the determinations of the inorganic carbon concentrations.

Further remarks:

At the end of the test the pH value of both inoculum blanks and the procedure control was 7.4, respectively. The pH values of the test units and the toxicity control were 7.3 and 7.5, respectively.

2.1.1.2 [Anonymous (2004)]

Study reference:

Anonymous (2004). TMPTA Determination of the Biodegradability in the CO2- Evolution test.

Detailed study summary and results:

Executive summary

The test substance was tested in the CO2 evolution test (OECD 301B) for biodegradability. Afetr 28 d biodegradation values of 70-80% CO2/ThOD were reached classifieng it as biodegradable (but failing the 10d-window) under aerobic environmental conditions.

Test type:

OECD Guideline 301 B (Ready Biodegradability: CO2 Evolution Test)

GLP compliant

Test substance:

2,2-bis(prop-2-enoyloxymethyl)butyl prop-2-enoate / 15625-89-5 / 239-701-3

EC number 239-701-3

CAS number 15625-89-5

IUPAC name: 2,2-bis(prop-2-enoyloxymethyl)butyl prop-2-enoate

Molecular formula (if other than submission substance): C15H20O6

- Molecular weight (if other than submission substance): 416 g/mol

- Physical state: liquid

- Impurities (identity and concentrations): acrylic acid, 80 mg/kg

- Purity test date: 86,5g/100g

Materials and methods:

Oxygen conditions

aerobic

Inoculum or test system

activated sludge, domestic, non-adapted

Duration of test (contact time): 28d

Initial test substance concentration : 33 mg/L test material

TC: 602 mg/l

Reference: aniline

Stocjk solution 393.1 mg/l

ThOC value: 304 mg/l

Inoculum: municipal STP: Lambsheim

Results:

Parameter

% degradation (CO2 evolution)

% Degradation of test substance: 70-80 after 28 d

Applicant's summary and conclusion

Validity criteria fulfilled : yes

Interpretation of results

readily biodegradable, but failing 10-day window

Conclusions

The test item reached in the CO2 evolution test (OECD 301B) degradation values of 70-80% CO2/ThOD classifieng it as biodegradable but failing the 10d-window.

2.1.2 Other degradability studies

2.1.2.1 [Anonymous (2010)]

Study reference:

Anonymous (2010). Photomer 4194 F, Hydrolysis as a Function of pH. Testing laboratory: Dr. U. NOACK-LABORATORIEN, Kaethe-Paulus Str. 1, 31157 Sarstedt, Germany. Owner company: PARAD Consortium REACHCentrum. Study number: CPH13611. Report date: 2010-09-01.

Detailed study summary and results:

Test type:

OECD Guideline 111 (Hydrolysis as a Function of pH)

GLP compliant

Test substance:

Name of test material (as cited in study report): Photomer 4149 F

CAS number: 28961-43-5

- Molecular formula: C21H32O9
- Molecular weight: 428.48 g/mol
- Physical state: Liquid, clear light yellowish
- Analytical purity: 100 %
- Purity test date: 2010-03-19
- Lot/batch No.: S700040031
- Expiration date of the lot/batch: 2011-01-04
- Stability under test conditions: Not specified
- Storage condition of test material: 6 +/- 2 °C, protected from moisture and light

Materials and methods:

Analytical monitoring

yes

Details on sampling

- Sampling intervals for the parent/transformation products: In the preliminary test, samples were taken at test start (0 h) and at test end (120 h). For definitive testing, samples were taken at test start and at a minimum of 8 spaced points, normally between 10 and 90 % of hydrolysis, at each test temperature.
- Sampling intervals/times for pH measurements: Test start
- Sampling intervals/times for sterility check: The sterility of the definitive test solutions for pH 7 and 9 was checked by colony forming units (CFU)-determination with Water Plate Count agar. The CFU was determined by incubation at 25°C for 48 hours.
- Sample storage conditions before analysis: All test item containing samples were analysed immediately (max. 1 % of total incubation time until start of analyses) via LC-MS.

Details on analytical methods

DETAILS ON PRETREATMENT

- Clean up method:e.g. chemical used for chemistry method (Cu, Hg,...) or phase and solvent used for

SPE method:

The samples were diluted with acetonitrile, dilution factor 1:2 within the test vessel. Afterwards the solution was diluted with 30 % acetonitrile : 70 % HPLC water with formic acid (0.1 %) (dilution factors 1:400, 1:200 and 1:100) and analysed.

IDENTIFICATION AND QUANTIFICATION OF PARENT COMPOUND

- Separation method (e.g. HPLC, GC): LC
- Conditions (column, mobile phase, etc.):

Column Nucleodur C18 Gravity 1.8 µm, 50 x 2.0 mm, batch 6033, MACHEREY-NAGEL

Column temperature 40 °C

Mobile phase Pump A: HPLC water + 0.1 % formic acid

Pump B: Methanol + 0.1 % formic acid

Gradient mode, see Table below.

Time[min] A[%] B[%]

0.00 50 50

0.20 50 50

1.70 10 90

2.00 10 90

2.01 50 50

3.00 50 50

Flow rate 0.55 mL/min

Run time 3 min

Injection volume 10 µL (full loop)

Detector Parameters

Detector Parameters for 2 EO, 3 EO, 4 EO and 5 EO

Compound m/z precursor ion [Da] m/z product ion [Da] Cone voltage[V] Collision energy[eV]

Primary Secondary Primary Secondary

2 EO 407.35 313.31 99.04 37 20 24

3 EO 451.42 379.26 99.05 41 28 30

4 EO 495.48 423.35 99.05 47 30 34

5 EO 539.48 467.37 99.04 53 34 38

CONDITIONS OF DETECTION

Type Multi Reaction Mode (MRM)

Ionisation mode Electro spray positive

Capillary voltage 0.30 kV

Source temperature 150 °C

Cone gas flow (N2) 30 L/h

Desolvation gas temperature 600 °C

Desolvation gas flow (N2) 1000 L/h

Dwell time 0.032 s

Collision gas pressure 3.14 x 10-3 mbar

- Detection method (e.g. ECD, UV, MS, ICP-AES, ICP-MS): MS-MS

- Detection limits (LOD, LOQ) (indicate method of determination/calculation):

Based on the calibration and the requirements of the study design, the limits of quantification (LOQ) of the analytical methods were fixed at 0.12 mg/L for method A and method B at pH 9 and 0.2 mg/L for method B at all pH values and checked by means of accuracy.

- Linearity: The analytical system gave linear response in the range of 5 – 150 µg Photomer 4149 F/L.

The linearity (R2) of the calibration curves was 0.992 for the components 2 EO, 3 EO, 4 EO and 5 EO for Photomer 4149 F

- Repeatability: 6 aliquots of the highest and lowest standard concentration prepared from a single homogeneous sample were analysed. Mean values, standard deviations and variations of coefficients were calculated.

Repeatability of Injections of Photomer 4149 F

Sum of peak areas of all components [mAU*s]; method B

Photomer 4149 F

Serial No. 5 µg/L 150 µg/L

Mean ± SD 4401 ± 53.7 129357 ± 440.3, CV [%] 1.22 0.34

Internal or external calibration: external

- Extraction recovery (indicate if results are corrected or not for recoveries):

Five replicates of buffer at pH 4, 7 and 9 fortified with 0.2 mg Photomer 4094 F/L (LOQ) and 2 mg Photomer 4094 F/L (10 x LOQ) and two buffer control samples each were prepared as described for the samples (total dilution factor of 100) and analysed. The mean recovery of the fortified samples should be between 70 and 110 %, ideally with the mean in the range 80 – 100.

Recovery Rates of Fortified Samples at pH 4, method A

Fortified concentrations: 0.12 mg/L (LOQ) and 1.2 mg/L (10 x LOQ)

Photomer 4149 F

1 x LOQ 10 x LOQ

Replicate

Meas. conc. RR Meas. conc. RR

[mg/L] [%] [mg/L] [%]

Mean 0.104 86 1.30 108

SD 0.00778 0.0246, CV [%] 7.50 1.89

Recovery Rates of Fortified Samples at pH 4, method B

Fortified concentrations: 0.2 mg/L (LOQ) and 2 mg/L (10 x LOQ)

Photomer 4149 F

1 x LOQ 10 x LOQ

Replicate

Meas. conc. RR Meas. conc. RR

[mg/L] [%] [mg/L] [%]

Mean 0.177 88 2.01 101

SD 0.00467 0.0162, CV [%] 2.64 0.81

Recovery Rates of Fortified Samples at pH 7, method A

Fortified concentrations: 0.12 mg/L (LOQ) and 1.2 mg/L (10 x LOQ)

Photomer 4149 F

1 x LOQ 10 x LOQ

Replicate

Meas. conc. RR Meas. conc. RR

[mg/L] [%] [mg/L] [%]

Mean 0.132 110 1.29 107

SD 0.00265 0.0177, CV [%] 2.01 1.38

Recovery Rates of Fortified Samples at pH 7, method B

Fortified concentrations: 0.2 mg/L (LOQ) and 2 mg/L (10 x LOQ)

Photomer 4149 F

x LOQ 10 x LOQ

Replicate

Meas. conc. RR Meas. conc. RR

[mg/L] [%] [mg/L] [%]

Mean 0.163 82 2.01 100

SD 0.00297 0.0153, CV [%] 1.82 0.76

Recovery Rates of Fortified Samples at pH 9, method A

Fortified concentrations: 0.12 mg/L (LOQ) and 1.2 mg/L (10 x LOQ)

Photomer 4149 F

1 x LOQ 10 x LOQ

Replicate

Meas. conc. RR Meas. conc. RR

[mg/L] [%] [mg/L] [%]

Mean 0.0839 70 1.14 95

SD 0.00851 0.0669, CV [%] 10.1 5.85

Recovery Rates of Fortified Samples at pH 9, method B

Fortified concentrations: 0.12 mg/L (LOQ) and 1.2 mg/L (10 x LOQ)

Photomer 4149 F

1 x LOQ 10 x LOQ

Replicate

Meas. conc. RR Meas. conc. RR

[mg/L] [%] [mg/L] [%]

Mean 0.118 98 1.24 103

SD 0.00261 0.0480, CV [%] 2.22 3.87

- Specificity: Response of blank values of control samples was significantly lower than 30 % of LOQ. The signals detected on the secondary ion trace confirmed the identity of all components for all analysed samples above LOQ level.

Buffers

- pH: pH 4, pH 7, pH 8

Type and final molarity of buffer:

Buffer solution pH 4 45 mL of 0.1 mol/L NaOH were mixed with 250 mL 0.1 mol/L KH2-Citrate and diluted to 500 mL with double distilled water.

Buffer solution pH 7 148.15 mL of 0.1 mol/L NaOH were mixed with 250 mL 0.1 mol/L KH2PO4, diluted to 500 mL with double distilled water.

Buffer solution pH 9 106.5 mL of 0.1 mol/L NaOH were mixed with 250 mL 0.1 mol/L H3BO3 in 0.1 mol/L KCL, diluted to 500 mL with double distilled water.

- Composition of buffer: Buffers were freshly prepared from chemicals with analytical grade or better quality. If necessary, volumes were adapted. Buffers were purged with nitrogen for 5 min. Then the pH of the test solution was checked to a precision of at least 0.1 at the required temperature and adjusted if necessary. Buffers were sterilised by filtration through 0.2 µm.

Details on test conditions

TEST SYSTEM

- Type, material and volume of test flasks, other equipment used: Sterile amber HPLC vials, volume 4mL
- Sterilisation method: Autoclaving of double distlled water, sterilisation of buffer solutions by filtration through 0.20 µm.
- Measures taken to avoid photolytic effects: Photolytic effects were avoided by using amber vials.
- If no traps were used, is the test system closed
- Is there any indication of the test material adsorbing to the walls of the test apparatus? No

TEST MEDIUM

- Volume used/treatment: 2 mL
- Kind and purity of water: see above
- Preparation of test medium: Solutions of the test item with a concentration of 10 mg/L were prepared in buffer solution pH 4, 7 and 9.

An aliquot of the respective sterile buffer was spiked with a test item stock solution. Aliquots of the test

solutions were filled into the vials, sealed and transferred into the thermostat. The time between test item application and transfer to thermostat/analysis did not exceed 30 min.

- Renewal of test solution: None

Duration of test

12.5 h, pH9, Temp. 50 °C Initial conc. Measured other: < LOQ

143 h, pH9, Temp. 30 °C Initial conc. Measured 0.33 mg/L

379 h, pH9, Temp. 20 °C Initial conc. Measured 0.9 mg/L

715 h, pH7, Temp. 30 °C Initial conc. Measured 8.03 mg/L

716 h, pH7, Temp. 20 °C Initial conc. Measured 8.94mg/L

714 h, pH7, Temp. 50 °C Initial conc. Measured 1.17 mg/L

Number of replicates

Duplicates, single injection

Negative controls

yes Sterile buffer solutions (pH 4, 7 and 9)

Statistical methods

The concentration of the test item was determined as a function of time. The log of the concentrations are plotted against time (log ct vs. t) and the slope of the resulting straight line from regression analysis gives the rate constant kobs [1/unit of time].

Due to an unsuitable positioning of the temperature sensor for the 50 °C thermostatisation system (waterbath) data gaps due to false negative readings of the automated temperature monitoring device were observed. For the effected testing conditions (pH 4 and 7 at 50 °C) all false negative readings were eliminated for temperature evaluation, resulting in monitoring gaps of up to 18 h. As for a period of over 700 h, with exception of the eliminated readings, no values outside the intended temperature range were observed, the temperature constancy over the whole study duration could be assumed.

Results

Preliminary study

In a preliminary test more than 10 % of the test item at pH 7 and 9 has been hydrolysed after 120 hours. Due to stability of the test item at pH 4, 50 °C over a period of 120 hours, the definitive study for pH 4 was aborted. The study was reclassified as preliminary test.

The used analytical method was method A.

Test performance

- Method validation
- Preliminary test (5 d)
- Preparation of the sterile test solutions
- Thermostatisation of the test solutions
- Analysis of samples
- Calculation of reaction rate constants and half lives for the test item

Transformation products

no

Dissipation DT50 of parent compound

pH4, 50°C, DT50 > 1year

Hydrolysis Results for the registered substance at pH 7 and 20 °C

Hydrolysis time [h]	Concentration [mg/L]	Log Concentration	
0.00	9.60	0.982	A
23.2	10.3	1.01	A
91.5	9.18	0.963	A
123	10.1	1.00	B
217	9.86	0.994	B
451	9.42	0.974	B
528	9.89	1.00	B
716	8.94	0.951	B

Hydrolysis Results for the registered substance at pH 7 and 30 °C

Hydrolysis Time [h]	Concentration [mg/L]	Log Concentration	Method
0.00	9.60	0.982	A
22.6	10.3	1.01	A
91.5	9.02	0.955	A
123	9.76	0.989	B
217	9.40	0.973	B
450	8.68	0.939	B
528	8.84	0.946	B
715	8.03	0.905	B

Hydrolysis Results for the registered substance at pH7 and 50°C

Hydrolysis Time [h]	Concentration [mg/L]	Log Concentration	Method	
0.00	9.60	0.982	A	
22.0	9.06	0.957	A	
41.4	12.8	1.11	A	1)
91.6	7.16	0.855	B	
123	7.08	0.850	B	
147	6.30	0.799	B	
216	5.20	0.716	B	
357	3.40	0.531	B	
528	1.92	0.283	B	
714	1.17	0.068	B	

Hydrolysis Results for the registered substance at pH 9 and 20 °C

Hydrolysis Time [h]	Concentration [mg/L]	Log Concentration	Method
0.00	10.1	1.00	A
22.0	9.10	0.959	A
39.8	7.20	0.857	A
90.4	4.96	0.695	B
121	3.98	0.600	B
146	3.96	0.598	B
193	3.12	0.494	B
259	1.71	0.233	B
379	0.900	-0.046	B

Hydrolysis Results for the registered substance at pH 9 and 30 °C

Hydrolysis Time [h]	Concentration [mg/L]	Log Concentration	Method
0.00	9.40	0.973	B
6.92	9.70	0.987	B
11.6	8.84	0.946	B
28.5	5.08	0.706	B
31.3	4.96	0.695	B
49.9	3.22	0.508	B
53.4	3.16	0.500	B
72.7	1.91	0.281	B
77.0	1.73	0.238	B
143.0	0.33	-0.481	B

Hydrolysis Results for the registered substance at pH 9 and 50 °C

Hydrolysis Time [h]	Concentration [mg/L]	Log Concentration	Method
0.00	9.40	0.973	B
1.98	6.50	0.813	B
4.23	5.14	0.711	B
6.27	3.54	0.549	B
8.02	2.36	0.373	B
9.52	1.80	0.255	B
10.6	1.55	0.190	B
11.9	1.25	0.0969	B
12.5	LOQ	LOQ	B

Overall remarks

For the testing condition pH 4, 50 °C no transformation was observed after 120 h of hydrolysis. Therefore, according to the guideline, no kinetic testing was deemed necessary and a half life of > 1 year, for environmental relevant temperatures, could be assumed. The p = 95 % confidence interval for the pH 7, 20 °C test condition could not be calculated, as the slope of the regression graph was not significantly non zero and therefore no intercept with the asymptotes of the confidence graphs, inside the definition range, could be calculated.

Reaction Rate Constants and Half Lives with Confidence Intervals of the registered substance

	pH 7	pH 9				
	20 °C	30 °C	50 °C	20 °C	30 °C	50 °C
Reaction rate constant kobs [1/s]	2.28 x 10-8	7.10 x 10-8	8.25 x 10-7	1.77 x 10-6	6.65 x 10-6	4.78 x 10-5
Confidence Interval of kobs [1/s]	Slope of regression graph not significantly non zero	3.62 x 10-8 to 1.03 x 10-7	7.99 x 10-7 to 8.56 x 10-7	1.60 x 10-6 to 1.97 x 10-6	6.11 x 10-6 to 7.18 x 10-6	4.40 x 10-5 to 5.26 x 10-5
Half life T½ [h]	8454	2711	233	109	28.9	4.03
Confidence Interval of T½ [h]	Slope of regression graph not significantly non zero	1873 to 5319	225 to 241	97.8 to 120	26.8 to 31.5	3.66 to 4.38
Half life T½ [d]	352	113	9.72	4.54	1.20	0.17

Conclusions

Reaction rate constants and half lives with their corresponding confidence intervals are given above. The registered substance F is seen to be slightly hydrolytically instable at pH 7, 20 °C and 30 °C and hydrolytically instable at pH 7, 50°C and pH 9, with increasing instability to higher pH and temperatu re. At pH 4 and 50 °C no hydrolysis within 120 h was observed and, according to the guideline, a half life of > 1 year could be assumed for environmental relevant temperatures.

2.2 Acute toxicity

2.2.1 Short-term toxicity to fish

2.2.1.1 [Anonymous (2016)]

Study reference:

Anonymous (2016). Trimethylolpropantriacrylate (CAS 15625-89-5) – Fish, acute toxicity test according to the OECD 203 guideline.

Detailed study summary and results:]

Test type:

OECD Guideline 203 (Fish, Acute Toxicity Test)

GLP compliant

Test substance:

2-ethyl-2-[[(1-oxoallyl)oxy]methyl]-1,3-propanediyl diacrylate / 2,2-bis(prop-2-enoyloxymethyl)butyl prop-2-enoate / 15625-89-5 / 239-701-3

EC number 239-701-3

CAS number 15625-89-5

IUPAC name 2,2-bis(prop-2-enoyloxymethyl)butyl prop-2-enoate

Test material form

solid - liquid: suspension

Details on test material

- Name of test material (as cited in study report): TMPTA
- Physical state: Clear, colorless liquid
- Analytical purity: 80.2 %
- Lot/batch No.: 41095133SP01
- Expiration date of the lot/batch: 30 September 2015
- Storage condition of test material: At room temperature protected from light

Materials and methods:

Test organisms (species) *Danio rerio*

Details on test organisms

The test organisms were Danio rerio (Teleostei, Cyprinidae), obtained on the 28 July 2016 from a recognised supplier: La Grande Rivière France (69490 Saint-Forgeux). All fish were in good health and free from any apparent malformation. Mortality of the batch was less than 5% in the week preceding the start of the study. No disease treatments were administered throughout holding and testing. Holding was maintained within the laboratory at a temperature of 21-25oC in glass tanks. A light cycle of 12 h light and 12 h dark was applied, illumination being provided by fluorescent tubes (intensity between 400 and 800 lux at the surface of the tanks). Tanks were aerated to ensure that the dissolved oxygen concentration is at least 60% of air saturation value in holding tanks and in test tanks. During holding, fish were fed twice per day with ground flake food TetraMin®. The fish were not fed for a period of 24h prior to test commencement or throughout the duration

of the test. Fish were added to the test tanks within 30 min after the completion of preparation of the test solutions. All test fish were weighed prior to the test and a representative number of test fish batch (10 at random) was measured after the test to assess compliance with guideline criteria.

Test type: semi-static
Water media type: freshwater
Limit test: yes
Total exposure duration: 96 h

Test conditions
Hardness: The total hardness was determined at 250 mg CaCO3/L
Test temperature: The temperature during the definitive test remained constant within ± 2°C during the test but was not maintained in a range from 21°C to 25°C: mean measured temperature: 21.1°C, min.: 20.0°C, max.: 21.8°C.
During the preliminary range-finding test the temperature was maintained in a range from 21°C to 25°C and remaining constant within ± 2°C during the test: mean measured temperature: 22.6°C, min.: 22.2°C, max.: 23.3°C.
pH: varied between 8.0-8.1. The pH of the old solution varied betwen 7.5-7.7.
Dissolved oxygen: varied between 99.2-100% saturation, and of the old solution varied between 82-92% saturation.
Salinity: Not measured
Conductivity : Not measured
Nominal and measured concentrations: the stability of the substance was not confirmed over the test period, the exposure concentrations were thus based on the geometric mean of measured concentrations.
Reference substance (positive control) : yes potassium dichromate
Number of fish in each test vessel: 5

Results:

Test solution (mg/L)	**Cumulative dead fish**					Mortality at the end of the period
	T0+3h	T24h	T48h	T72h	T96h	
Control	0	0	0	0	0	0.0%
0.21	0	0	0	0	0	0.0%
0.47	0	0	0	0	0	0.0%
1.03	0	0	0	0	0	0.0%
2.27	0	0	2	2	5	100.0%

5.00	0	5	5	5	5	100.0%

Key results:

96h-NOEC 0.89 mg/L

96h-LOEC 1.71 mg/L

96h-LC10 0.75 mg/L

96h-LC50 0.87 mg/L

Results with reference substance (positive control)

The sensitivity of the test system and the methodology are evaluated with every batch of fish by per forming an acute toxicity test on potassium dichromate. The values of 24h-LC50 obtained with the ba tch used for the definitive test were as follows: 24h-LC50 = 173.6 mg/L (obtained on 11 August 2016)

Applicant's summary and conclusion

Validity criteria fulfilled

yes i)The mortality in the control did not exceed 10% at the end of the test; ii)The dissolved oxygen in the test tanks remained above 60% of the air saturation value at the end of the test; iii)The pH did not vary by more than 1 unit.

Conclusions

The acute toxicity of the test substance to Danio rerio exposed under semi-static conditions for 96h was assessed according to the OECD 203 Guideline. Fish were exposed to a series of test solutions renewed every day throughout the test period

2.2.1.2 [Anonymous, 1988]

Study reference:

Anonymous (1988). Report on the study of acute toxicity. Test substance: Trimethylolpropantriacrylat, Species: Golden orfe (Leuciscus idus).

Detailed study summary and results:

Test type:

EU Method C.1 (Acute Toxicity for Fish), DIN 38412/15

No GLP

Test substance:

Trimethylolpropantriacrylat / Trimethylolpropantriacrylat

Details on test material

- Name of test material (as cited in study report): Trimethylolpropantriacrylat

- Other: solubility in water: 5 g/l

- Lot/batch No.: Tank 1 (Dec. 10, 1987)

Materials and methods:

Test organisms (species): Leuciscus idus

Test type: static

Water media type: freshwater

Limit test: yes

Total exposure duration: 96h

Analytical monitoring: not specified

Reasons for the selection of the concentrations: Based on the results of 2 range finding studies (LC50 after 96h: between 0.3 and 1 mg/l) the concentrarions , spaced by a factor of 2.2 were fixed as follows: 0.1, 0.215, 0.464, 1 and 2.15 mg/l

Preparation of test substance: The product dissoled in acetone (1g/100 ml) was added to the test water. Subsequently the fish were placesd into the aquaria. Analogously to the highest amount of solvent used, 0.215 ml acetone/l test water was added to the control aquarium.

Photoperiod: 16 h light 8 h darkness.

Fish:

supplier: Fish hatchery Paul Eggers, 2354 Hohenwestedt, Germany

Body length : 8.1cm (range: 7.2 -9.3cm)

Body weight: 5.3 g (range: 3.6 - 8.3g)

Test vessels: glass aquarium 30cm x 22cm x 24cm

Volume of water : 10 l

Loading g fish/l test water) : 5.-3

withdrawal of food before exposure: 1d

Results

Key result

LC50 = 1.47 mg/L (nominal) based on mortality

NOEC: 1 mg/l

max concentratiuon causing no mortality: 1 mg/l minimum

concentration causing 100% mortality: 2.15 mg/l

				mortality			
nominal conc. [mg/l]	Number of fish	1h	4h	24h	48h	72h	96h

0.100	10	0	0	0	0	0	0
0.215	10	0	0	0	0	0	0
0.464	10	0	0	0	0	0	0
1.000	10	0	0	0	0	0	0
2.150	10	0	0	10	10	10	10
0.000	10	0	0	0	0	0	0
solvent control	10	0	0	0	0	0	0

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

The LC 50 in Leuciscus idus of the test substance was determined to be 1.47 mg/l.

Executive summary

Trimethylolpropantriacrylat was tested for acute fish toxicity in Leuciscus idus according to DIN 38412/15. After 96 h exposure to nominal concentrations of 0.1,0.215, 0.464, 1 and 2.15 mg/l an LC 50 was determined to be 1.47 mg/l as the geometric mean of the highest concentration with no mortality, i.e. 1 mg/l, and the concentration with 100% mortality, i.e. 2.15 mg/l.. The NOEC was 1 mg/l.

2.2.2 Short-term toxicity to aquatic invertebrates

2.2.2.1 [Anonymous (1991)]

Study reference:

Anonymous (1991). Bestimmung der akuten Wirkung von Trimethylolpropantriacrylat auf die Schwimmfähigkeit des Wasserflohs Daphnia magna (Straus).

Detailed study summary and results:

Test type:

EU Method C.2 (Acute Toxicity for Daphnia)

GLP compliance : not specified

Test substance:

EC number EC name

239-701-3 EC Inventory

CAS number CAS name

15625-89-5

IUPAC name

2,2-bis(prop-2-enoyloxymethyl)butyl prop-2-enoate

Name of test material (as cited in study report): Trimethylolpropantriacylat

- Substance type: Acrylate
- Physical state: fluid
- Analytical purity: 90%
- Impurities (identity and concentrations): higher molecular addition products
- Lot/batch No.: Tank

Materials and methods:

No analytical monitoring

Test organisms (species) : Daphnia magna

Test type: static

Water media type: freshwater

Total exposure duration : 48h

Overall remarks

Temperature: 20 ± 1 °C

test vessels: test tube with max. vol 20ml

test volume: 10 ml

age of daphnia : 2 -24 h

density (daphnia/ml): 1/2

daphnia /test tube: 5

replica /test concentration: 4

replica /control: 4

day/night period: 16h/8h

Results:

LC50 = 19.9 mg/L, nominal test material based on mortality.

Any other information on results

after 6 h

EC0 = 100 mg/l

EC50 = > 100 mg/l

EC100 = > 100 mg/l

after 24h

EC0 = 25 mg/l

EC50 = 52.9 mg/l

EC100 = > 100 mg/l

after 48 h

EC0 = 12.5 mg/l

EC50 = 19.9 mg/l

EC100 = 100 mg/l

Determination of the EC50 values followed the Probit method according to Finney.

For EC0 the highest tested concentration was taken at which an effect <= 10% occurred.

For EC100 the lowest tested concentration was taken at which an effect of 100% occurred.

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

The test substance's acute toxicity to Daphnia was determined to be 19.9 mg/l.

Executive summary

The test substance was tested for aquatic toxicity against Daphnia magna according to the EU Method C.2 (Acute Toxicity for Daphnia). After 48h exposure the aquatic toxicity was determined to be: EC0 = 12.5 mg/l, EC50= 19.9 mg/l and EC100= 100 mg/l. Based on the test substance is considered to be moderately toxic to daphnia.

2.2.2.2 [Anonymous (1988)]

Study reference:

Anonymous (1988). Determination of the acute toxicity of Trimethylolpropantriacrylat to the waterflea Daphnia magna Straus.

Detailed study summary and results:

Test type:

EPA OPP 72-2 (Aquatic Invertebrate Acute Toxicity Test)

No GLP

Test substance:

EC number EC name

239-701-3 EC Inventory

CAS number CAS name

15625-89-5

IUPAC name

2,2-bis(prop-2-enoyloxymethyl)butyl prop-2-enoate

Materials and methods:

Test organisms (species): Daphnia magna

Test type: static

Water media type: freshwater

Total exposure duration : 48h

Results:

LC50 = 19 mg/L nominal, test material based on mobility.

Any other information on results

after 48 h:

EC0 = 12.5 mg/l

EC50 =18.99 mg/l

EC100 = 50 mg/l

Applicant's summary and conclusion

Executive summary

In a test following the guideline "Guideline for testing of chemicals, EG-1 of Jan. 1982, issued by the EPA, office of toxic substances" the EC50 of Daphnia magna (Straus) against Trimethylolpropantriacrylat (90% purity) was determined, using Dimethylsulfoxid (DMSO) as a solvent. After 48h the following results were obtained: EC0: = 12.5 mg/l, EC50 = 18.99 mg/l and the EC100 = 50 mg/l. Based on the test results Trimethylolpropantriacrylat was evaluated to be moderately toxic for Daphnia magna.

2.2.3 Algal growth inhibition tests

2.2.3.1 [Anonymous (1989)]

Study reference:

Anonymous (1989). Inhibition of the algae cell multiplication according to DIN 38412 L9.

Detailed study summary and results:

Test type:

EU Method C.3 (Algal Inhibition test)

GLP compliant

Test substance:

Details on test material

- Name of test material (as cited in study report): Trimethylol propane triacrylate

Materials and methods:

Test organisms (species)

Desmodesmus subspicatus (previous name: Scenedesmus subspicatus)

Study design

Test type: static

Water media type: freshwater

Limit test: yes

Total exposure duration: 96h

test system: Scenedesmus subspicatus

origin: Algensammlung , Göttingen SAG 86.81

test concentrations:

1, 2.5, 5, 10 , 25 , 50 mg/l

frequency and duration of administration: 1x4 replicates, 96h

measureing method: fluorometric (impulse flurometer)

spectrophotometric scan: 300 -780 nm

Statistical method: Tallaria , R. J. Jakob, L.S (1979): The Dose-Response Relation in Pharmacology pp.98 - 103, Publisher: Springer

Results:

Effect concentrations:

	72 h			96 h		
	[mg/l]	P 95%		[mg/l]	P 95%	
EBC 10 :	.605	.295	1.240	.573	.205	1.597
EBC 50 :	7.157	5.108	10.027	4.859	2.960	7.977
EμC 10 :	1.900	.710	5.083	2.184	.870	5.482
EμC 50 :	18.849	8.090	43.915	14.535	6.768	31.215

Cell density

Concentration		0h	24h	48h	72h	96h
[mg/l]		N/ml x 103	N/ml x 103	N/ml x 103	N/ml x 103	N/ml x 103
control		12	26	98	301	547
1		12	26	83	221	407
2.5		12	26	74	212	375
5		12	26	64	180	300
10		12	24	54	128	189
25		12	20	36	60	64
50		12	17	21	21	17

Evaluation after 72h

Concentration	Growth rate	Biomass inhibition [%]	Rate-related inhibition [%]
[mg/l]			
control	1.07	--	--
1	0.97	20.4	9.6
2.5	0.96	27.0	10.9
5	0.90	37.5	16.0
10	0.79	51.5	26.5
25	0.54	73.1	50.1
50	0.19	88.5	82.6

Evaluation after 96h

Concentration	Growth rate	Biomass inhibition [%]	Rate-related inhibition [%]
[mg/l]			
control	0.95	--	--
1	0.88	25.6	7.7
2.5	0.86	30.1	9.9
5	0.80	41.7	15.7
10	0.69	59.6	27.8
25	0.42	82.7	56.2
50	0.09	95.1	90.9

after 72 h:

EBC10: 0.605 mg/l EuC10: 1.9 mg/l

EBC50: 7.157 mg/l EuC50: 18.849 mg/l

after 96 h:

EBC10: 0.573 mg/l EuC10: 2.184 mg/l

EBC50: 4.859 mg/l EuC50: 14.535 mg/l